University of Minho
School of Sciences

Inês Marques Machado

Synthesis of catecholic amino acids and peptides or conjugates of catechols with amino acids and peptides

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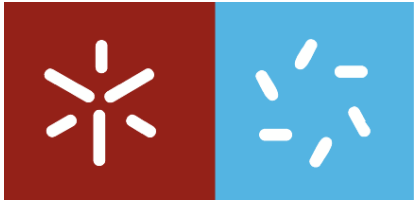

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Synthesis of catecholic amino acids and peptides or conjugates of catechols with amino acids and peptides

Master's Dissertation in Medicinal Chemistry

Supervisor:
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STATEMENT

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Abstract

Oxidative stress is an imbalance between the amount of oxidants and antioxidants in favour of the oxidants. Thus, when there is excessive production of reactive oxygen species (ROS) or antioxidant defence mechanisms are diminished, oxidative stress occurs. In order to maintain normal cellular functioning any excess of ROS must be continuously inactivated through the use of antioxidants.[1,2]

Phenolic amino acids, such as tyrosine, exhibit a wide range of biological activities and have shown to be effective antioxidants in different in vitro antioxidant activities studies. Phenolic acids coupled with amino acids are involved in suppressing the adverse effects of oxidative stress and have a wide range of biological activities, such as antioxidant,[3] anticancer[4] and antimicrobial.[5]

In this work a strategy based on the Ugi reaction was developed for the synthesis of new phenolic amino acids. Hydroxybenzaldehyde derivatives were reacted with 4-methoxybenzylamine, cyclohexyl isocyanide and benzoic acid, phenolic acids or catecholic acids to give Ugi adducts that were treated with trifluoroacetic acid, yielding *N*-acyl (hydroxyphenyl)glycine amides in good yields. The use of *N*-benzyloxycarbonylglycine as acid component allowed the preparation of a 3,4-dihydroxyphenylglycyl dipeptide derivative. Radical-scavenging activity studies of the polyphenolic amino acid derivatives showed a sharp increase in activity with the increase in number of hydroxyl or catechol groups present.

Several studies suggest that a cocktail of antioxidants, endowed with different mechanisms of action, results more effectively than a single antioxidant because of the synergistic effect of the molecules. To promote possible synergistic mechanisms and to better understand the mechanistic aspects, the use of modified and/or dualistic molecules is a valuable approach.[6]

Dehydroamino acids constitute an important class of non-proteinogenic amino acids with various biological activities, including antioxidant. *N*-phenolic and *N*-catecholic derivatives of dehydrophenylalanine were synthesized using a method which comprises coupling phenolic or catecholic acids and/or phenolic or catecholic amines.

Resumo

O stress oxidativo é um desequilíbrio entre a quantidade de oxidantes e antioxidantes em favor dos oxidantes. Assim, quando há uma produção excessiva de espécies reativas de oxigénio (ROS) ou os mecanismos de defesa antioxidante estão diminuídos, ocorre stress oxidativo. Para manter o funcionamento celular normal, qualquer excesso de ROS deve ser continuamente inativado através da utilização de antioxidantes.[1,2]

Aminoácidos fenólicos, como a tirosina, exibem uma ampla gama de atividades biológicas e mostraram-se antioxidantes eficazes em diferentes estudos de atividade antioxidante *in vitro*. Os ácidos fenólicos acoplados a aminoácidos estão envolvidos na supressão dos efeitos adversos do stresse oxidativo e possuem uma ampla gama de atividades biológicas, como antioxidante,[3] anticancerígeno[4] e antimicrobiano.[5]

Neste trabalho foi desenvolvida uma estratégia baseada na reação de Ugi para a síntese de novos aminoácidos fenólicos. Os derivados de hidroxibenzaldeido foram feitos reagir com 4-metoxibenzilamina, isocianeto de ciclohexilo e ácido benzóico, ácidos fenólicos ou ácidos catecólicos para dar origem a aductos Ugi que foram tratados com ácido trifluoroacético originando amidas *N*-acil hidroxifenilglicina com bons rendimentos. A utilização de *N*-benziloxicarbonilglicina como componente ácido permitiu a preparação de um derivado dipeptídico de 3,4-*di*-hidroxifenilglicil. Estudos de atividade de eliminação de radicais dos derivados de aminoácidos polifenólicos mostraram um aumento acentuado na atividade com o aumento do número de grupos hidroxilo ou catecol presentes.

Vários estudos sugerem que, devido ao efeito sinérgico das moléculas, um cocktail de antioxidantes dotados de diferentes mecanismos de ação, resulta mais eficazmente do que um único antioxidante. Para promover possíveis mecanismos sinérgicos e compreender melhor os aspetos mecanísticos, o uso de moléculas modificadas e/ou dualísticas é uma abordagem valiosa.[6]

Desidroaminoácidos constituem uma classe importante de aminoácidos não proteinogénicos com várias atividades biológicas, incluindo antioxidante. Derivados *N*-fenólicos e *N*-catecólicos da desidrofenilalanina foram sintetizados usando um método que compreende o acoplamento de ácidos fenólicos ou catecólicos e/ou aminas fenólicas ou catecólicas.

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ABTS	2,2′-Azino-bis-(3-ethyl-benzothiazoline-6-sulfonate)
ACAT	Acyl-CoA:cholesterol acyltransferase
AOC	Antioxidant capacity
Boc	*tert*-Butyloxycarbonyl group
Boc_2O	*tert*-Butyl pyrocarbonate
br. s	Broad singlet
δ	Chemical shift in parts per million
CAT	Catalase
d	Doublet
Δaa	α,β-Dehydroamino acids
DOPG	3,4-dihydroxy phenyl glycine
DPPH	2,2-diPhenyl-1-picryl hydrazyl
DMAP	4-Dimethylaminopyridine
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulfoxide
GPx	Glutathione peroxidase
GSH	Glutathione
HBTU	Hexafluorophosphate benzotriazole tetramethyl uronium
HOBt	Hydroxybenzotriazole
Hz	Hertz
J	Coupling constant (Hz)
L-DOPA	L-3,4-diHydroxyphenylalanine
LDL	Low-density lipoprotein
LP	Lipid peroxidation
m	Multiplet
MCR	Multicomponent reactions
min	Minute(s)
mol	Mole(s)
M.p.	Melting point
NADPH	Nicotinamide adenine dinucleotide phosphate

NEt_3	Triethylamine
NMR	Nuclear magnetic resonance
o	*ortho*
ORAC	Oxygen radical absorbance capacity
p	*para*
ppm	Parts per million
QSAR	Quantitative structure–activity relationship
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
s	Singlet
SOD	Superoxide dismutases
t	Triplet
TEAC	Trolox equivalent antioxidant capacity
TFA	Trifluoroacetic acid
TFE	Trifluorethanol
TLC	Thin layer chromatography
TMG	*N,N,N',N'*-Tetramethylguanidine

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1. Introduction

1.1. Oxidative stress and antioxidants

1.1.1 Oxidative stress

Oxidative stress is the term that refers to the imbalance between the formation of free radicals, namely, reactive oxygen (ROS) and nitrogen (RNS) species and the activity of the antioxidant defences.[1] Free radicals are chemical species containing an unpaired electron. They are highly unstable, with extremely short half-life, very reactive and are constantly being formed in the human body.

Most biological molecules are nonradicals that contain only paired electrons. Once radicals form, they can react either with another radical or with another molecule by various types of interactions. The rate and selectivity of these types of reactions depends on high concentrations of the radicals, on delocalization of the single electron of the radical (thus increasing its life time), and on the absence of weak bonds in any other molecules present with which the radical could interact.[8]

Free-radical mechanisms have been implicated in the pathology of several human diseases. Oxidative stress, resulting from an exaggerated production of ROS/RNS or from reduction of mechanisms of antioxidant defences, has been implicated in the development of many neurodegenerative diseases (Figure 1) such as Parkinson and Alzhemier's disease and is also responsible for aging, atherosclerosis, rheumatoid arthritis and carcinogenesis.[9] Therefore, ROS/RNS production must be controlled in order to maintain normal cellular function. Any excess must be continuously inactivated by antioxidant enzymes or by using synthetic antioxidants.[8]

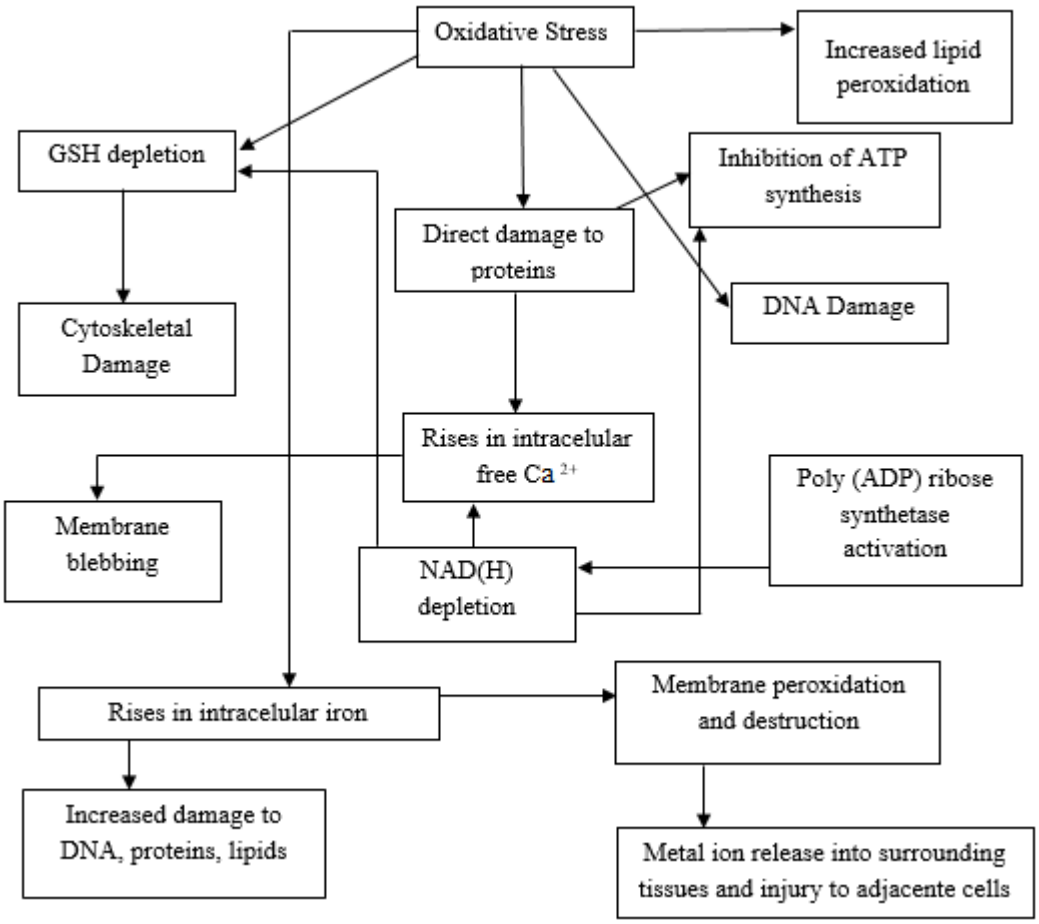


Figure 1 – Mechanisms of cellular damage in oxidative stress (adapted from reference 8).

Antioxidant defences remove $O_2^{\cdot -}$ and H_2O_2. Superoxide dismutases (SOD) removes $O_2^{\cdot -}$ by greatly accelerating its conversion to H_2O_2. Catalases in peroxisomes convert H_2O_2 into water and O_2 and help to dispose of H_2O_2 generated by the action of the oxidase enzymes that are located in these organelles. Other important H_2O_2-removing enzymes in human cells are the glutathione peroxidases.[8]

When produced in excess, ROS can cause tissue injury. However, tissue injury can itself cause ROS generation, which may (or may not, depending on the situation) contribute to a worsening of the injury. Assessment of oxidative damage to biomolecules by means of emerging technologies based on products of oxidative damage to DNA (e.g., 8-hydroxydeoxyguanosine), lipids (e.g., isoprostanes) and proteins (altered amino acids) has advanced the understanding of the underlying mechanisms. This assessment also facilitates supplementation and intervention studies designed and conducted to test antioxidant efficacy in human health and disease.[8]

Oxidative stress can have natural causes or unnatural causes. Natural causes occur in situations of extreme physical exercise or in inflammatory processes. Unnatural causes result, for example, from the presence of xenobiotics in the body or from radioactive environments.[10]

The chemical reactions that result from attack of ROS upon proteins are complex. Free-radical attack upon proteins generates radicals from amino acid residues, and electrons can be transferred between different amino acids (Figure 2). The levels of any one (or, preferably, of more than one) of these products in proteins could in principle be used to assess the balance between oxidative protein damage and the repair of (or, more likely, hydrolytic removal of) damaged proteins.[8]

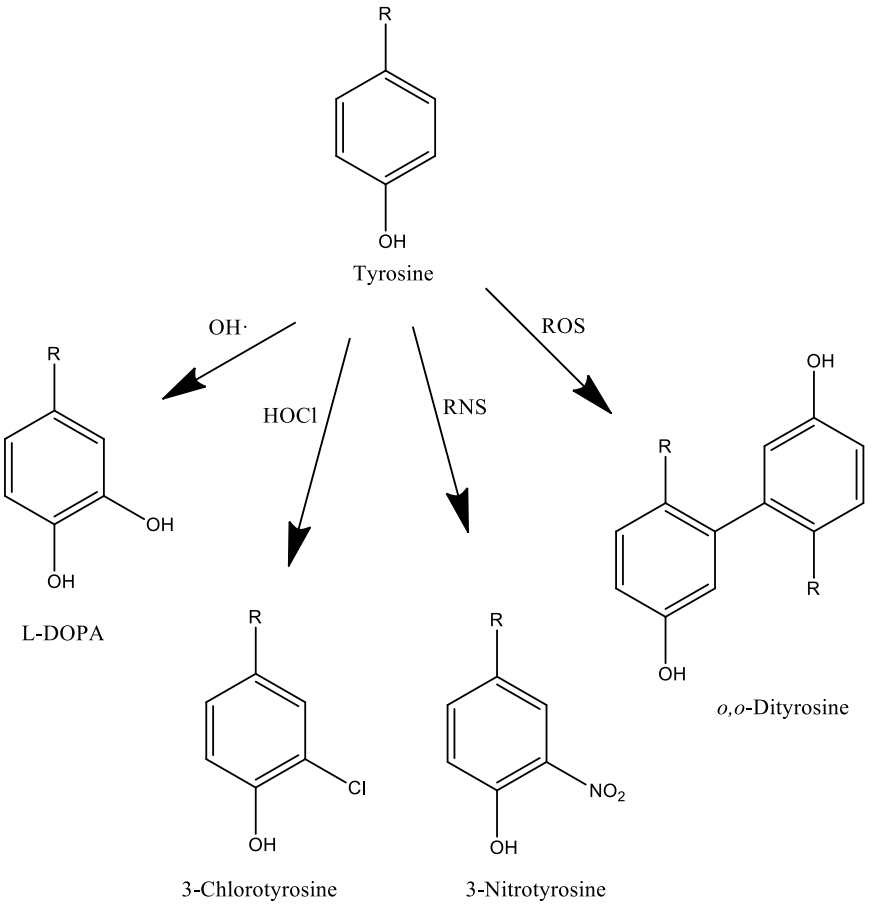


Figure 2 – Products of oxidative protein damage (adapted from reference 8).

1.1.2. Antioxidants

The free-radical reaction of lipid peroxidation, characterized as the oxidative deterioration of lipids containing any number of carbon-carbon double bonds, is an important issue in the food industry. Manufacturers minimize oxidation in lipid-containing foods by use of antioxidants during the manufacturing process; foods are produced that maintain their nutritional quality over a defined

shelf life. By contrast, biomedical scientists and clinicians are interested in antioxidants because they protect the body against damage by reactive oxygen species.[8]

The body's exposure to free radicals from various sources has led the body to develop a number of defence mechanisms to eliminate these free radicals. Our antioxidant defence system includes endogenous antioxidants (enzymatic and non-enzymatic) and exogenous antioxidants, with the diet being the main source (Table 1). Endogenous and exogenous antioxidants act synergistically to maintain or re-establish redox homeostasis. An example is the regeneration of vitamin E by glutathione (GSH) or vitamin C to prevent lipid peroxidation processes, which can affect membrane fluidity and damage membrane proteins by e.g., inactivating receptors, enzymes and ion channels, even disrupting membrane integrity resulting eventually in cell death.[11]

Table 1 – Human antioxidant defence systems.[11]

<table>
<tr><th colspan="2">Antioxidant defence system</th></tr>
<tr><th>Endogenous antioxidants</th><th>Exogenous antioxidants</th></tr>
<tr><td>Enzymatic antioxidants:
• Superoxide dismutase (SOD): enzyme detoxifying superoxide radical ($O_2^{\cdot-}$)
• Catalase (CAT) and glutathione peroxidase (GPx): enzymes involved in the detoxification of peroxides (CAT against H_2O_2, and GPx against both H_2O_2 and ROOH)
• Glutathione reductase: enzyme involved in the regeneration of glutathione
• Thioredoxin reductase: enzyme involved in the protection against protein oxidation
• Glucose-6-phosphate dehydrogenase: enzyme involved in the regeneration of NADPH</td><td rowspan="2">Principal dietary antioxidants from fruits, vegetables and grains
• Vitamins: vitamin C, vitamin E
• Trace elements: zinc, selenium
• Carotenoids: b-carotene, lycopene, lutein, zeaxanthin
• Phenolic acids: chlorogenic acids, gallic acid, cafeic acid, etc.,
• Flavonols: quercetin*, kaempferol*, myricetin*
• Flavanols: proanthocyanidins and catechins
• Anthocyanidins: cyanidin* and pelagonidin*
• Isoflavones: genistein*, daidzein* and glycitein*
• Flavanones: naringenin*, eriodictyol* and hesperetin*
• Flavones: luteolin* and apigenin*</td></tr>
<tr><td>Non-enzymatic antioxidants (principal intracellular reducing agents):
GSH, uric acid, lipoic acid, NADPH, coenzyme Q, albumin, bilirubin</td></tr>
</table>

*and their glucosides.

The biological concept of antioxidant refers to any compound capable of inhibiting or retarding the oxidation of an oxidizable substrate. Antioxidants reduce oxidative stress and the associated diseases.[8]

Antioxidants that can directly neutralize ROS and terminate free radical mediated oxidative reactions have beneficial activities in protecting the human body from many chronic diseases. Also, antioxidants effectively retard the onset of lipid peroxidation by lowering the concentration of free radicals. However, an antioxidant that neutralizes free radicals usually becomes a radical species and can also be harmful.[12]

Some antioxidants have toxicity and to prevent this, Bast and Haenen put forward a few points to follow when directly involved in natural product research and processing.[13] These include: (1) an urgent change in the approach used to evaluate food additives and supplements to include arbitrary safety factors which allows extrapolation of research results from rat to human, (2) understanding of the bio-kinetics, bioavailability and biotransformation of food and drug ingredients should be extended, (3) biokinetic/bio-efficacy modelling should be used to help in standardizing dosages, and (4) the risk/benefit analyses of all supplements that have health claims should be warranted. High-dose antioxidant supplements do no good and obviously cause harm to human health while low dose mixtures can sometimes be good but may be recommended only for undernourished populations.[14]

Because some synthetic antioxidants cause health risks, namely in the liver, and/or are related to carcinogenesis, the search for natural antioxidants has been greatly intensified in recent years. Among the various natural antioxidants, phenolic compounds are reported to be active, quenching oxygen derived free radicals by donating a hydrogen atom or an electron to the free radical.[14] The catechol group normally imparts antiradical activity to molecules, being present in several antioxidants, as is the case of catecholamines and flavonoids. These antioxidants are capable of neutralizing reactive species.[15]

1.2. Antioxidant amino acids

1.2.1. Proteinogenic amino acids

Natural phenols represent a wide class of compounds endowed with interesting biological activities. Phenolic compounds are widely studied as antioxidants because these compounds have a stable structure after the free radicals are quenched.[9] Phenolic amino acids can be obtained from natural sources or synthetically.[9]

An example of a natural phenolic amino acid is tyrosine. Studies have shown L-tyrosine to be an effective antioxidant in different in vitro antioxidant activity assays.

In a study on the antioxidant capacity (AOC) of amino acids and their derivatives, carried out by Torkova *et al.*, experimental data on antioxidant properties were obtained in vitro.[16] The peroxyl radical and the 2,2′-azino-bis-(3-ethyl-benzothiazoline-6-sulfonate) (ABTS) radical cation and are widely used in vitro to characterize natural antioxidants. The corresponding assays are known as oxygen radical absorbance capacity (ORAC) and trolox equivalent antioxidant capacity (TEAC), respectively. The authors concluded that in the ORAC assay, the AOC decreased in a row Trp > Tyr > Met = Cys > His. Only Cys, Tyr and Trp exhibited antioxidant activity in the TEAC assay in concentrations <1 mM, due to the lower reactivity of the ABTS cation-radical compared with the peroxyl radical. Thus, Tyr and Met demonstrated dissimilar tendencies in TEAC and ORAC assays: while Tyr exhibited the highest AOC against peroxyl radical and low AOC against ABTS, Met possessed no activity in the ORAC assay and was rather active against ABTS.[16]

In order to study the influence of tyrosine C- and N-terminal modifications on its AOC, the authors investigated the antioxidant properties of tyrosine dipeptides with aliphatic amino acids. In the TEAC assay, the average AOC values for dipeptides with N- and C-terminal tyrosine were 4.81 ± 0.10 and 1.70 ± 0.27 μmol TE/μmol (micromoles of Trolox equivalents per micromole of sample), respectively. Dipeptides containing the N-terminal tyrosine residue exhibited, on average, 1.4 times higher AOC values than free tyrosine (3.4 μmol TE/μmol) (Figure 3). Dipeptides with C-terminal Tyr exhibit half the AOC values compared with free tyrosine. This testifies to the important role of a free tyrosine amine group, the presence of which, apparently, determines the mechanism of interaction of the tyrosine residues with cation-radical ABTS and the set of resulting products. In the ORAC assay, the AOC values of tyrosine dipeptides with aliphatic amino acids was practically no different from free tyrosine AOC.[16]

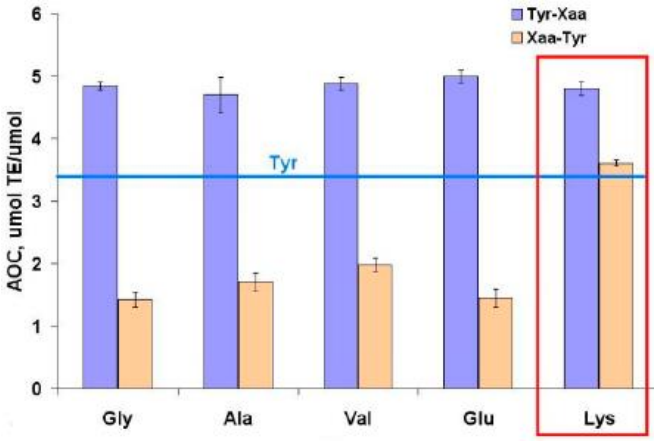


Figure 3 – Antioxidant capacity of tyrosine dipeptides in TEAC assays (the red squares indicate peculiar behaviour of dipeptides with ionogenic side chains).[16]

The transmembrane domains of integral membrane proteins show an outstanding accumulation of tyrosine residues, especially in the region of highest lipid density, which comprises the inner portion of the lipid head groups and the beginning of the hydrocarbon tails. These residues perform vital antioxidant functions inside lipid bilayers and protect cells from oxidative destruction.[14] Long-chain acylated tyrosines are potent inhibitors of lipid peroxidation and oxidative cell death. The antioxidant functions of tyrosine may provide a specific explanation for their unique transmembrane distribution pattern and the high vulnerability of low-protein neuronal membranes to oxidative stress, as seen in neurodegenerative disorders.[17]

According to Moosmann and Behl,[17] the cytoprotective antioxidant effects of membrane-anchored tyrosine may be explained by the special capability of the phenolic group to serve as hydrogen radical donor inside lipidic phases, thereby interfering with peroxidizing free radical chain reactions. Tyrosine residues themselves are converted to relatively stable and nonreactive phenoxyl radicals, a mechanism which has been used to rationalize the antioxidant effects of a wealth of nonpeptide low molecular mass antioxidants, among them estrogen, serotonin, and tocopherol. Chemically, phenoxyl radicals are much more stable and have longer lifetimes than simple peroxyl radicals, so any reverse reaction or the propagation of the radical-mediated peroxidizing chain reaction are inhibited. By being radicalized, tyrosine becomes more polar than their nonradicalized precursors, which may promote the preferred protrusion of these residues into zone 1 of the lipid bilayer, to come into contact with aqueous reducing molecules such as ascorbate or glutathione.[17]

Low-protein membranes and artificially composed lipid-only membranes are much more vulnerable to oxidants and this is attributed to lack of membrane proteins and thereby of tyrosine; for instance, mitochondrial inner membranes with their high protein content are much more resistant to oxidative stress than myelin sheaths with their low protein content. This may explain the unique vulnerability of low membrane protein cell types in cases of oxidative degenerative diseases.[17]

In conclusion, the accumulation of tyrosine in transmembrane proteins protects the surrounding lipid bilayer from peroxidation. Thus, tyrosinyl lipids may constitute a novel class of pharmacologically useful cytoprotective antioxidants.[17]

Tyrosine is also responsible for the formation of L-3,4-dihydroxyphenylalanine (L-Dopa). L-Dopa is the immediate precursor of the natural neurotransmitter dopamine and is widely used as medication in Parkinson's disease, to alleviate the symptoms due to decreased dopamine levels in the brain. In addition, many studies show that L-Dopa antioxidant activity plays an important role

in the reduction of chronic diseases, mutagenesis and carcinogenesis by preventing H_2O_2-induced oxidative damage to cellular DNA.[18]

The biosynthesis of L-Dopa from tyrosine starts by addition of a second hydroxyl group to the aromatic ring by the enzyme tyrosine hydroxylase. L-Tyrosine hydroxylation is the committed step in the synthesis of catecholamines and is subject to feedback inhibition by the end products. Subsequently, L-Dopa, can be decarboxylated by aromatic-L-amino acid decarboxylase to form dopamine.[19]

Studies have shown L-Dopa to be an effective antioxidant in different in vitro antioxidant activity assays. Its activity is comparable to standard antioxidant compounds, such as butylated hydroxyanisole, butylated hydroxytoluene, α-tocopherol and trolox, a water-soluble analogue of tocopherol. Also, L-Dopa demonstrated higher antioxidant and radical scavenging activities than L-tyrosine. Thus, the antioxidant activities seem to be highly controlled by the number of phenolic hydroxyl groups.[12]

1.2.2. Non-proteinogenic amino acids

Non-coded amino acids can have a variety of applications such as antiviral, antitumor, anti-inflammatory or immunosuppressor compounds, in structure-activity relationship studies or in the development of new biomaterials.[20-22] On several occasions unnatural α-amino acids have been used to modify the conformation and/or stability of a biologically active peptides. The pharmacological properties of a peptide are a function of its conformation, which in-turn is dictated by the amino acid composition and sequence. In this respect, unusual amino acids are useful for carrying out more systematic studies (e.g., QSAR). For example, incorporation of α-aminoisobutyric acid into oligopeptides is known to rigidify the peptide backbone through the formation of β-turns or α-helices.[21] Therefore, incorporation of these amino acids into peptides and proteins is a good way to obtain compounds with new chemical, structural and pharmacological properties.

1.2.2.1. Phenolic amino acids

1.2.2.1.1. Hydroxyphenylglycine

The non-proteinogenic amino acid *p*-(hydroxyphenyl)glycine is found in several peptidic natural products including the vancomycin group of antibiotics (e.g. vancomycin, chloroeremomycin, A47934 and complestatin) as well as other antimicrobial compounds such as ramoplanin. The *p*-(hydroxyphenyl)glycine residues play crucial roles in the structure and function of these antibiotics and of the final glycopeptide antibiotic. In vancomycin and chloroeremomycin, the D-*p*-

(hydroxyphenyl)glycine residue at position four of the heptapeptide is oxidatively cross-linked with the aryl rings of halogenated β-hydroxytyrosines at positions two and six, creating part of the rigid scaffold characteristic of these antibiotics (Figure 4).[23]

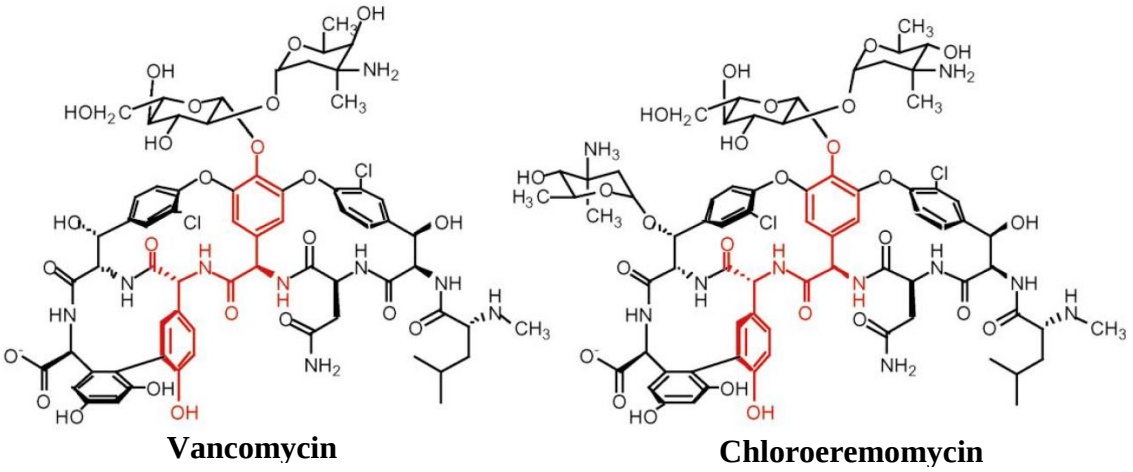


Figure 4 – Chemical structures of representative *p*-(hydroxyphenyl)glycine (highlighted in red) containing peptidic natural products.[23]

The committed synthesis of *p*-(hydroxyphenyl)glycine and the clustering of the enzymes for its proposed biosynthetic pathway with other enzymes for vancomycin assembly, suggests this residue provides reactivity and/or architecture not available with the proteinogenic homolog tyrosine.[19]

Proposals for *p*-(hydroxyphenyl)glycine biosynthesis for vancomycin group antibiotics have been based on labelling studies where either [^{13}C]tyrosine or [^{2}H,^{13}C]tyrosine was fed to growing cultures. Analysis of the final glycopeptide product showed incorporation of the labelled residues as D-*p*-(hydroxyphenyl)glycine residues, leading to the conclusion that these are derived from tyrosine.[24]

The strongest in vivo support for this proposed *p*-(hydroxyphenyl)glycine biosynthetic pathways comes from [^{14}C]tyrosine incorporation into the antibiotic nocardicin A (Figure 5). In this L-lactam antibiotic, two molecules of *p*-(hydroxyphenyl)glycine are incorporated into the final product.[23]

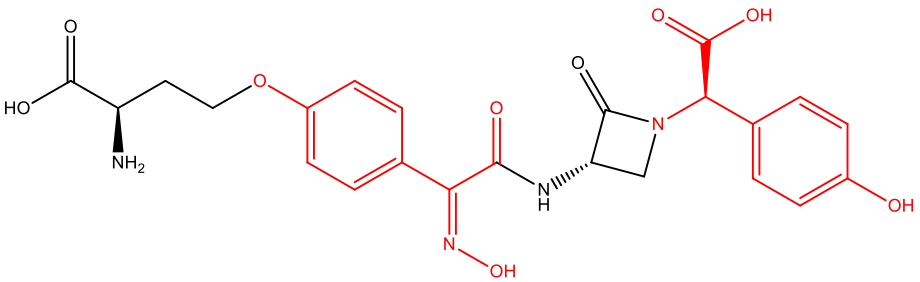


Figure 5 – Structure of nocardicin A.

1.2.2.1.2. Di(hydroxyphenyl)glycine

Catechols are benzene derivatives with two neighbouring (*ortho*-) hydroxyl groups that occur ubiquitously in nature. For example, catecholamine neurotransmitters, such as adrenaline and noradrenaline, fulfil essential and well-defined biochemical roles. Catechol derivatives can also be found as active species in a variety of environments, displaying a remarkable degree of chemical and physico-chemical versatility that has already inspired, and is still leading to, an ever-growing number of applications as functional materials.[25]

Despite the diverse properties of cathecolic compounds, little attention has been given to amino acids with the *ortho*-dihydroxyaryl function, such as 3,4-dihydroxyphenylglycine (DOPG). The chemical synthesis of 3,4-dihydroxyphenylglycine has been accomplished by Strecker's synthesis and demethylation reactions using as starting material 3,4-dimethoxybenzaldehyde and its enzymatic oxidation by tyrosinase studied.[26] Tyrosinase is a copper-containing enzyme widely distributed in nature. It catalyses the hydroxylation of monophenols to *ortho*-diphenols and the oxidation of the latter to *ortho*-quinones using molecular oxygen.[27]

Synthetic 3,4-dihydroxyphenylglycine was used as a substrate for mushroom tyrosinase.[26] UV and visible spectral studies during the enzymatic oxidation of 3,4-dihydroxyphenylglycine showed that tyrosinase readily oxidized DOPG to the expected quinone. The glycyl-*o*-benzoquinone thus formed was highly unstable like its higher analogue, dopaquinone. However, unlike its counterpart, it failed to exhibit intramolecular cyclization reactions. Rather, glycyl-*o*-benzoquinone exhibited facile transformation to ultimately generate 3,4-dihydroxybenzaldehyde. Thus, it is assumed that 3,4-dihydroxyphenylglycine cannot undergo intramolecular Michael 1,4-addition like dopaquinone, due to the unfavourable positioning of the amino group.

Gąsowska *et al.* conducted a similar study with amino-(3,4-dihydroxyphenyl)methyl phosphonic acid, the phosphonic analogue of 3,4-dihydroxyphenylglycine, which is a potent inhibitor of tyrosinase. This compound turned out to be a substrate and was oxidized to *o*-quinone, which evolved to a final product identified as 3,4-dihydroxybenzaldehyde, the same as for 3,4-di(hydroxyphenyl)glycine.[27]

3,4-Dihydroxyphenylglycine has also been studied as copper ligand. Stereochemical considerations are thought to have a large bearing on the identity and strength of the complexes and complex polymers formed when these species involve simultaneous chelation of both sites of the ligand to metal ions. Such effects are very apparent in comparisons of the polymerization schemes of 3,4-dihydroxyphenylglycine (DOPG) and L-β-(3,4-dihydroxyphenyl)alanine (DOPA). The

two ligands are very similar, but DOPG has a side chain shorter by one -CH_2- group, and is, therefore, a less flexible molecule which leads to markedly different polymerization reactions. Thus, when the polymerization of the DOPA-copper system was compared to that of DOPG, closed ring dimerization does not occur, a difference attributable to the lower flexibility of the side-chain of this catecholamine.[28]

Based on the results obtained titrations at 1:1 molar ratio of DOPG to copper showed the formation of the 1:1 amino acid-copper type complex.[24] The authors thus proposed the formation of a DOPG-Cu(II) tetramer (Figure 6).

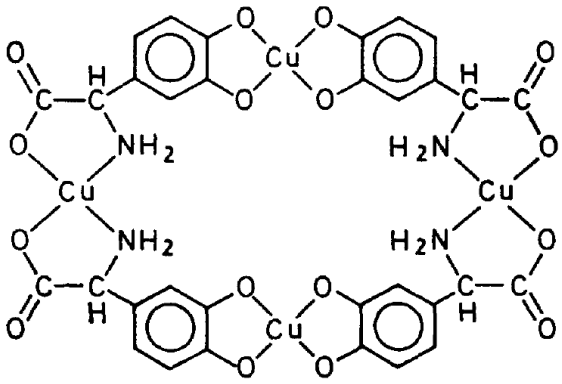
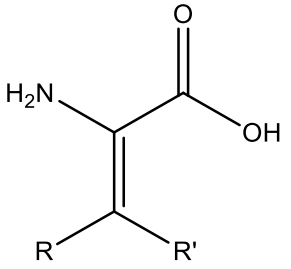


Figure 6 – Probable DOPG-Cu(II) tetramer.[28]

1.2.2. Dehydroamino acids

α,β-Dehydroamino acids (Δaa) are non-proteinogenic amino acids that contain a double bond between the α carbon and the β carbon (Figure 7). Dehydroamino acids constitute an important class of non-proteinogenic amino acids with various biological activities.[2,7,8,29-38] They are key intermediates in the synthesis of new amino acids and peptides, being found in several natural antibiotics and also play an important role in the active centre of some enzymes.[39]

The most common α,β-dehydroamino acids are dehydroalanine (ΔAla), dehydroaminobutyric acid (ΔAbu), dehydrophenylalanine (ΔPhe), dehydrovaline (ΔVal) and dehydroleucine (ΔLeu) (Figure 7).[40]



R= H, R'=H, **ΔAla**
R= CH_3, R'=H, **ΔAbu**
R= Ph, R'=H, **ΔPhe**
R= CH_3, R'=CH_3, **ΔVal**
R= $CH(CH_3)_2$, R'=H, **ΔLeu**

Figure 7 – Structure of the most common α,β-dehydroamino acids.

Due to the existence of the double bond that gives them a planar geometry and allows the existence of E/Z isomerism, dehydroamino acids have properties different from their saturated equivalents. Dehydroamino acids are not free in nature because they are very unstable and can undergo hydrolysis. They appear in some bacteria, yeasts and natural antibiotics.[40]

Dehydroamino acids activities include antioxidant properties, functioning as radical scavengers since they form stabilized free radical adducts, react with, and scavenge oxygen and hydroxyl radicals.[8] In a study realized by Suzen *et al.*, eleven *N*-acetyl dehydroalanine derivatives (*N*-acetyl DHA) were synthesised to evaluate their antioxidant properties on rat liver lipid peroxidation (LP) levels and 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging activity.[9] In order to identify the possible structure - antioxidant activity relationships and to guide in the design of new analogues, several dehydroalanine derivatives in the form of amides with aliphatic or cyclic chains were studied (Figure 8). Compounds **c**, **f** and **j** slightly scavenged the level of DPPH radical by about 27, 46, and 56%, respectively. However, compounds **a**, **d**, **e**, **f**, **g** and **h** showed a strong inhibitory effect on LP and the inhibition was in the range 76–90%. Compounds **b**, **c** and **i** also inhibited LP by about 54%, 58% and 66%, respectively but compounds **j** and **k** had no inhibitory LP activity at the same concentrations. The authors concluded that *N*-acetyl DHA derivatives which are substituted with aliphatic (up to 3 or 4 carbons) and cyclic side chains (5 membered) have significant hydroxyl radical scavenging activity.[9]

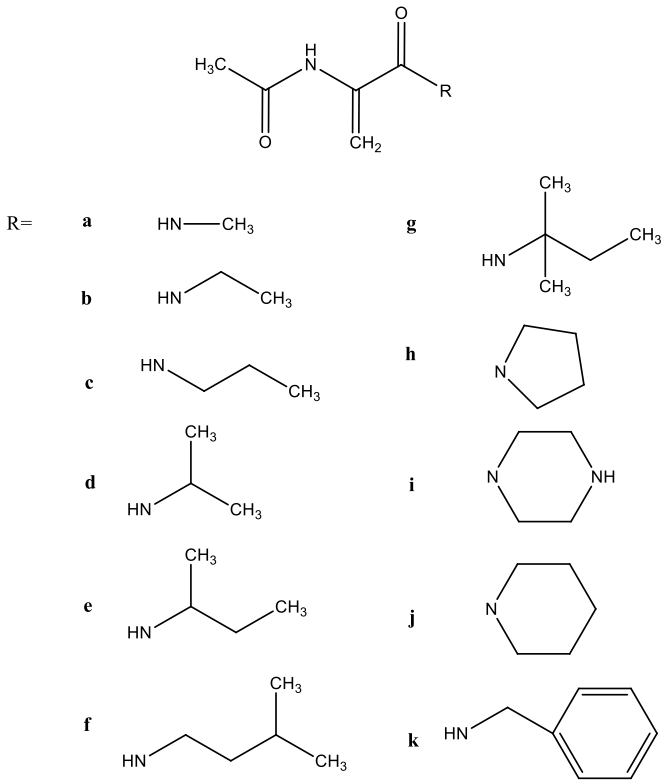


Figure 8 - *N*-Acetyl dehydroalanine derivatives (adapted from reference 9).

Continuing their study on the antioxidant activity of dehydroalanine, Suzen *et al.* studied various *N*-substituted dehydroalanine derivatives (Figure 9) using DPPH and superoxide ($O_{2}^{\cdot-}$) radical scavenging activity assays, and the in vitro effects on rat liver lipid peroxidation levels.[2] The results obtained where compared to α-tocopherol as reference. α-Tocopherol is a powerful antioxidant that is beneficial in the treatment of many free radical related diseases.[9]

No significant results were obtained with the DPPH and superoxide radical scavenging activity assays. However, all the compounds were effective in the LP experiment, allowing the conclusion that these dehydroalanine derivatives are able to scavenge $OH^{\cdot}$ radical, rather than DPPH or superoxide radicals. These result were similar to the earlier findings, in which the *N*-acetyl dehydroalanine derivatives showed a strong inhibitory effect on lipid peroxidation, while they had very little or no effect on DPPH radical.[9]

These studies led the authors to conclude that dehydroalanine derivatives, which are reactive Michael acceptors, can be active *in vitro* against oxidants.

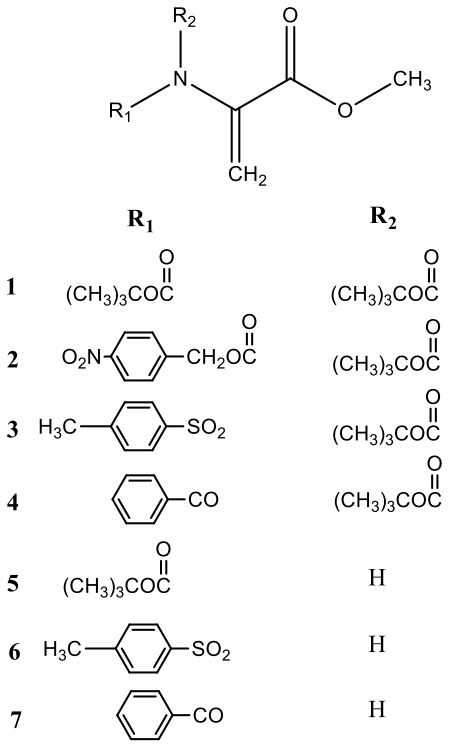


Figure 9 – Methyl esters of *N*-substituted dehydroalanine (adapted from reference 2).

1.3. Phenolic acids coupled with amino acids

Several studies suggest that a cocktail of antioxidants, endowed with different molecular structures and mechanisms of action, result more effective than a single antioxidant, due to the synergic effect between different types of molecules.[6,41-45]

Silvia *et al.* proposed the synthesis of a new set of molecular combinations of phenolic acid-amino acid and of phenolic acid-amino acid-dopamine conjugates.[46] The aim was to investigate the influence of the different phenolic moieties (in particular caffeic acid and 3,4-dihydroxyphenylacetic acid) on the antioxidant activity of the resulting molecules and thus improve the antioxidant activity of natural amino acids. It was shown that for significant antioxidant activity at least two hydroxyl moieties on the aromatic ring are necessary, as well as a spacer between the aromatic ring and the carboxylic acid. The trimer compounds phenolic acid-amino acid-dopamine, showed no significant improvement of the antioxidant activity due to the presence of the two additional OH groups from the dopamine moiety. This latter finding suggests that also the shape and complexity of the molecule may play a role and that there is a "saturation" on the maximum potency achievable by this approach.[46] The results obtained confirm that, molecular combinations improve

the antioxidant efficiency of natural antioxidants, this effect being designated by the author as the Centaurus tactic.

1.3.1. Phenolic acids coupled with proteinogenic amino acids

Phenolic acids coupled with amino acids or amines can be obtained from natural sources such as, fruits, vegetables and beverages or synthetically. It is assumed that these bioactive substances are involved in suppression of deleterious effects of oxidative stress and have a wide range of biological activities such as antioxidant,[3,47-50] anticancer[4] and antimicrobial.[5, 51-55] In fact, the accumulation of hydroxycinnamic acid amides constitutes part of the defence system of plants that is activated as a response to various environmental stimuli such as wounding, fungal infection or heavy metal ions.[56-59]

To find more active antioxidants with the hydroxycinnamoyl moiety, Wei Q-Y *et al.*[3] synthesized a series of *N*-hydroxycinnamoyl amino acid esters and evaluated their antioxidative activities by 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging and human red blood cells haemolysis methods. It was found that:

- *N*-caffeoyl amides exhibited the highest DPPH scavenging activities, whereas *N*-feruloyl amides demonstrated the highest antihaemolysis activities among the three different hydroxycinnamamides studied (caffeoyl, feruloyl, and *p*-coumaroyl);
- hydroxycinnamoyl amides were more effective than their corresponding free acid and ester compounds.

Thus, they concluded that the synthetic amide analogues of hydroxycinnamic acids exhibit stronger antioxidative activity than their corresponding free acids and esters.

This stronger antioxidative activity of linked phenolic compounds could be indirectly found in a study by Brand-Williams *et al.* These authors found a low antioxidant efficiency of the monohydroxylated compounds namely, phenol, coumaric acid, vanillin and vanillic acid. This may be explained by the presence of an electron withdrawing group (CHO or COOH) or, as is the case of phenol, the absence of any electron donating group. This poor aromatic ring resonance of the phenoxyl radical considerably lowers the antiradical efficiency.[60]

The observation that amides bearing *ortho*-methoxyl group vicinal to the hydroxyl functionality exhibit markedly higher antihaemolytic activities gave useful information for antioxidant drug design.[61]

Lee *et al.* prepared *N*-(4-hydroxycinnamoyl)-L-phenylalanine methyl ester (compound **1**, Figure 10), *N*-(3,4-dihydroxyhydrocinnamoyl)-L-aspartic acid dibenzyl ester (compound **2**, Figure 10), and

N-(3,4-dihydroxyhydrocinnamoyl)-L-alanine methyl ester (compound **3**, Figure 10) and evaluated their biological activities on lipoprotein metabolism.[61]

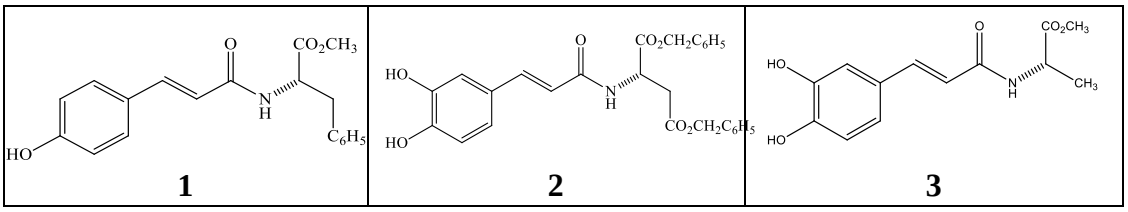


Figure 10 – Structure of *N*-(hydroxycinnamoyl) amino acid derivatives (adapted from reference 61).

These authors found that compounds **1** and **2** inhibited human acyl-CoA:cholesterol acyltransferase (ACAT) activities. Compounds **1** and **2** also served as antioxidants against copper mediated low-density lipoprotein (LDL) oxidation. Compound **3** showed a potent in vivo activity, with significant reduction of atherosclerotic lesion formation in hypercholesterolemic rabbit without improvement of serum lipid profile. These results suggest that the new cinnamic acid derivatives possess useful biological activity as anti-atherosclerotic agents.[61]

Another application of phenolic or catecholic amino acids is in the design of new peptide hydrogels that mimic mussel adhesive proteins. The extraordinary ability of mussel adhesive proteins is mainly attributed to the reversible metal-catechol coordination between metals like Fe^{3+} and catechol groups from the amino acid 3,4-dihydroxyphenyl-*L*-alanine (DOPA), and also to other interactions such as cation-p interactions.[62]

Mussel adhesion protein–inspired polymer research is motivated by the idea that the exceptional adhesive properties exhibited by the native proteins can be captured in synthetic polymer systems. A variety of chemical approaches may be employed, giving rise to linear or branched polymers functionalized with Dopa, Dopa peptides, or their catechol mimetics. These polymers provide simple platforms for studying the role of Dopa in mussel adhesion, as well as novel materials for wet adhesion. One of the compelling uses for these materials is in medical adhesion and sealing, in which catechols enhance adhesion to wet tissue surfaces. Progress is being made towards the development of mussel mimetic polymers capable of repairing both soft and hard tissues. Finally, there is rapid adoption of mussel mimetic polymers in the coatings field. Here, the adhesive qualities of the catechol may be important for anchoring polymers, biomolecules, and other compounds onto solid surfaces. With careful design of polymer composition and architecture, these coatings can give rise to adhesive or antiadhesive coatings.[63]

1.3.2. Phenolic acids coupled with dehydroamino acids

Due to the antioxidant properties of dehydroamino acids, their combination with phenolic or catecholic moieties can be a valuable approach for the development of new biologically active compounds.

In order to explore the previously proposed "Centaurus tactic", Monteiro *et al.*[64] combined the dehydroamino acid moiety with phenolic or catecholic acids. Thus, an innovative strategy for the synthesis of a series of *N*-phenolic and *N*-catecholic dehdroalanine and dehydrophenylalanine derivatives was developed. This methodology was applied with *N*-phenolic or *N*-catecholic serine or phenylserine methyl esters to give the corresponding dehydroalanine or dehydrophenylalanine derivatives (Figure 11).

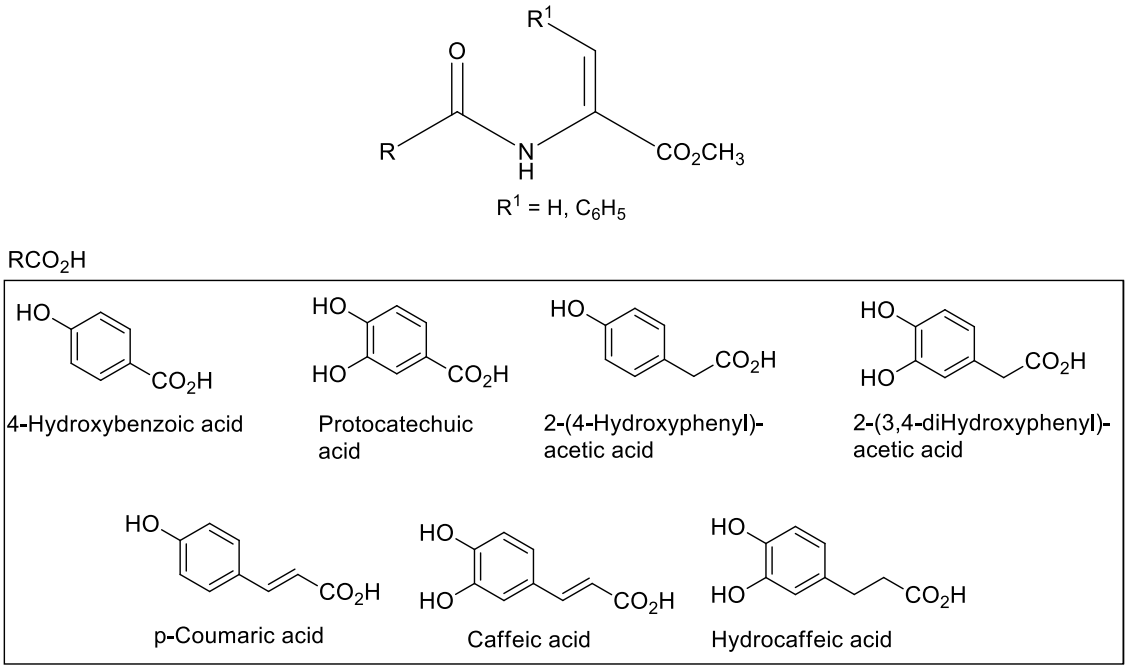


Figure 11 – Methyl esters of *N*-phenoyl and *N*-catechoyl dehydroalanine and dehydrophenylalanine (adapted from reference 64).

The toxicity of these dehydroalanine and dehydrophenylalanine derivatives was evaluated against a panel of human cancer and non-cancer cell lines. Preliminary assays on the toxicity on gastric cancer (AGS) and lung cancer (A549) cell lines, showed that the most potent molecules were the dehydrophenylalanine derivatives, which can be attributed to their higher liposolubility. Assays of these compounds on cell viability against a non-cancer cell line [lung fibroblasts (MRC-5)], showed that the molecules display very low or no toxicity. Thus, the toxicity of the molecules was higher towards cancer cells than non-cancer cells.[64]

1.4. Synthetic methodologies

1.4.1. Multicomponent reactions

In chemical reactions, usually only one or two chemical compounds participate. If more than two adducts are converted into products, usually such synthesis requires sequences of chemical reactions. Typically, after each step the intermediate or final product must be isolated and purified. The more steps needed, the more preparative work must be accomplished and with each step the yield of the final product decreases.

One-pot reactions that form products from three or more starting compounds are called multicomponent reactions (MCR).[65] The reagents may be different individual molecules, or they may be different functional groups present in the same reagent.

MCRs may be older than life. It is assumed, that adenine, one of the nitrogenous bases of DNA and RNA, prebiotically originates from five molecules of hydrogen cyanide. Note that each one of those five molecules plays a different role.

MCRs allow a great variety of products to be formed in a higher yield than by conventional multistep synthesis. Usually the starting materials are readily available or can easily be prepared.[66]

The first important MCR was discovered in 1850 by Strecker.[67] It is a three-component condensation of ammonia, an aldehyde, and hydrogen cyanide, leading to α-cyano amines, which can easily be hydrolysed to α-amino acids. In 1890, Hantsch discovered that α-chloro oxo compounds, ammonia, and 1,3-dioxo compounds condense to form pyrrol.[68] Bignelli introduced a three component dihydropyrimidine synthesis in 1893.[69] The best known MCR is Mannich's three component condensation (M-3CC) in which an amine and formaldehyde combine to form a Schiff base (imine) which subsequently reacts with an α-acidic reagent.[70] In 1921, Passerini described one of the most important reactions of isocyanides, a three-component condensation (P-3CC), in which they are reacted with a carboxylic acid and an oxo compound.[71] Bucherer and Bergs were the first to describe a MCR of four compounds (BB-4CC) in 1933.[72] Hydroformylation, is a very important MCR, described in 1948 by Roelen.[73] Asinger found a general thiazolidine synthesis in 1958. Two equivalents of an aldehyde or ketone react with sulphur and ammonia (A-3CC), and, alternatively, an α-halo oxo compound, ammonia, an oxo compound, and sodium hydrogen sulphide react to form a thiazolidine (A-4CC).[74] In 1959, Ivar Ugi investigated an efficient procedure, which allows the simultaneous joining of 4 different fragments: an isocyanide, an aldehyde, an amine and a carboxyl acid giving rise to α-acetamido carboxamides. (U-4CC).[75] The last important

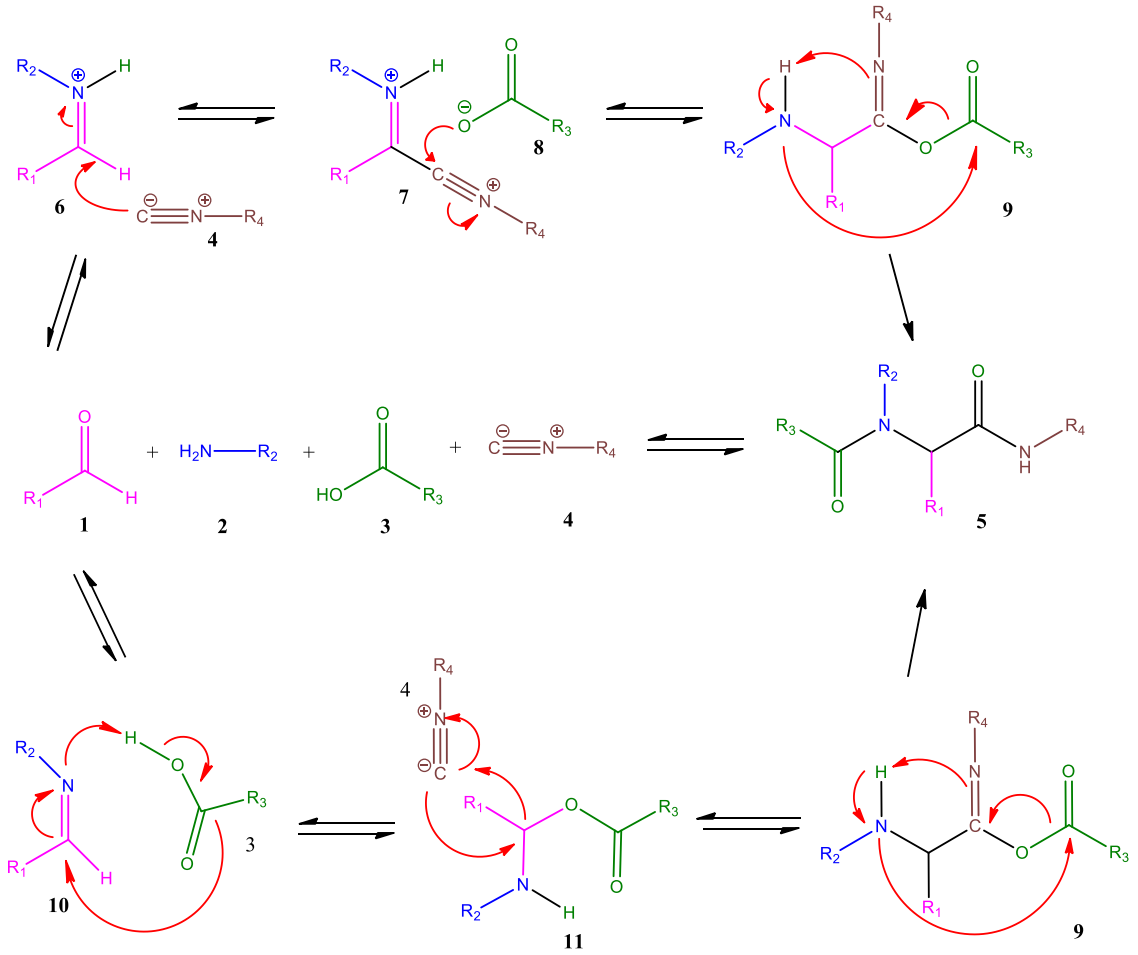
MCR is the Pausen-Khand reaction, published in 1971, where substituted cyclopentenones are easily accessible by this three-component reaction involving carbon monoxide, an alkyne, and an alkene.[76]

Multicomponent reactions have emerged in the last 20 years as a powerful tool in drug discovery. They are intrinsically endowed with high economic advantages and operational simplicity, being perfectly suited for rapid generation of libraries resulting from the high diversity of inputs.[77]

1.4.1.1. Ugi reaction

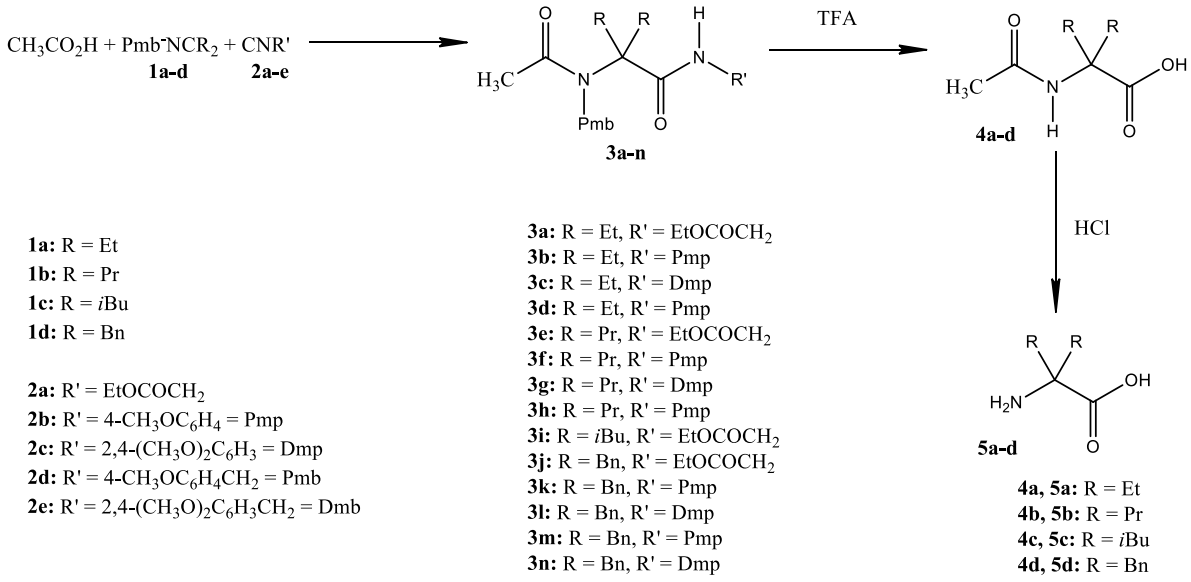
One important multicomponent reaction is the Ugi reaction (U-4CC), first reported by Ivar Ugi, in 1959 in which the condensation of an aldehyde, an amine, a carboxylic acid and an isocyanide occurs in a one-pot procedure. The Ugi reaction has allowed the preparation of molecules with high diversity. It is a valuable tool for generating α-amino acid derivatives in a very straightforward manner.

Two possible mechanisms for the Ugi reaction have been postulated (Scheme 1). In both mechanisms, the first step involves condensation of aldehyde **1** and amine **2** to give an imine that is protonated by the carboxylic acid **3**. The debate is whether the next step involves introduction of the carboxylic acid to **10**, causing isocyanide **4** to react with **11** via a S_N2 mechanism, or whether the isocyanide **4** first undergoes nucleophilic addition to imine **6**, followed by the addition of carboxylate **8** to **7**. Experiments supporting the formation of intermediate **7** versus **11** have not been performed.[78]



Scheme 1 – Postulated mechanism of the U-4CC (adapted from reference 78).

A strategy based on the modified Ugi reaction using as amine component 4-methoxybenzylamine and several isocyanide derivatives, followed by treatment of the adducts with trifluoroacetic acid (TFA) has been explored [79] This method enabled the synthesis of several *N*-acetylamino acids derivatives (compounds **4a-d**, Scheme 2). Schiff bases were prepared by azeotropic reflux of 4-methoxybenzylamine and diethyl, dipropyl, diisobutyl or dibenzyl ketone in toluene. The required isonitriles were prepared from the corresponding formamides. The Schiff bases were combined with the isonitriles and acetic acid in dry methanol and allowed to react at room temperature for one to two weeks. The expected Ugi–Passerini adducts, were obtained, usually in high yields independently of the bulkiness of the reagents or that of the reaction products. The Ugi–Passerini adducts were reacted with neat trifluoroacetic acid under reflux to remove the 4-methoxylbenzyl group at the amine function. The hydrolysis of the *N*-acetylamino acids with hydrochloric acid gave the corresponding free derivatives or their hydrochlorides in excellent yields (Scheme 2).



Scheme 2 – Synthesis of α,α-dialkylglycines by the Ugi–Passerini reaction (adapted from reference 79).

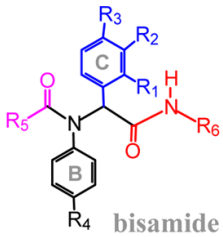
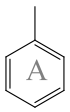
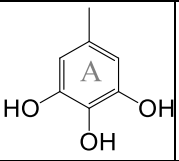
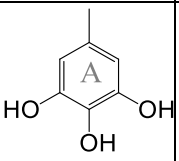
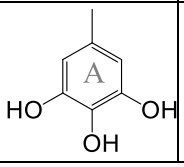
The authors demonstrated that neither the bulkiness of the dialkyl glycine side chains nor the nature of the isonitrile moiety seems to affect the lability of the amide bond at the C-terminus, thus allowing good cleavage selectivity and yields in all cases. Thus, the choice of the isonitrile should be based only on the availability and price of the starting material. In addition, the use of 4-methoxybenzylamine seems to be a good choice to allow the easy removal of the *N*-alkyl group present in the Ugi–Passerini adduct. Their results suggest that this is the best route for the synthesis of bulky α,α-dialkylglycines, as it involves simple procedures in clean reactions, leading to high overall yields, usually above 60%.[79] This procedure using as aldehyde component phenolic or catecholic benzyladehydes and as acid component phenolic or catecholic acids, could be an efficient approach for the synthesis of novel *N*-phenoyl or *N*-catechoyl (hydroxyphenyl)glycines.

1.4.1.2. Synthesis of polyphenolic amino acids by Ugi reaction

A simple approach to obtain conjugates of phenolic acids with (hydroxyphenyl)glycines would be a strategy where simple natural phenols are joined together in a one-step economical way. If hydroxyl-substituted benzoic acid, benzaldehyde, aniline, or isocyanides are employed in the Ugi reaction, the bis-amide obtained may be a suitable substrate for exploring the mutual antioxidant effects among phenolic hydroxyl groups at different positions. Reports on the application of Ugi reaction to construct antioxidants are not usually found, and the influence of the isocyanide moiety on antioxidant effectiveness remains unclear.[80] Wang and Liu investigated the mutual antioxidant effects of hydroxyl groups attached to different benzaldehyde and carboxylic acid components, as

well as the influence of the isocyanide component, on the antioxidant effect of bis-amides obtained by Ugi condensation. Some of the evaluated compounds are present in Table 2.

Table 2 – Structure of bis-amides obtained by Ugi condensation (adapted from reference 80).

	Comp.	R_1	R_2	R_3	R_4	R_5	R_6
	1	H	H	H	H		$-CH_2COOC_2H_5$
	2	H	H	H	H		$-CH_2COOC_2H_5$
	3	H	H	H	H		$-CH_2COOC_2H_5$
	4	H	H	H	H		$-C(CH_3)_3$
	5	H	H	H	H		

For quenching DPPH and galvinoxyl radical, the rate constant (*k*) values of compounds **3**, **4** and **5** are higher than those of other equivalent compounds (**1** and **2**). This shows that the 1,2,3-trihydroxyl groups attached to phenyl group A is the major functional group contributing to radical scavenging activity. Furthermore, by the comparison of *k* values of compounds **3**, **4** and **5**, it is found that the ferrocenylmethyl group enhances the *k* value of compound **5** markedly. However, in a comparison of abilities of these compounds to reduce the radical (quenching $ABTS^{\bullet+}$), the ferrocenylmethyl group enhances the *k* value of compound **5** not very significantly. The increasing effect of the isocyanide moiety on the antioxidant property follows the sequence ferrocenylmethyl group > *tert*-butyl group > ethoxycarbonyl group. Hence, the hydrogen atom donating ability is mainly due to the hydroxyl group attached to phenyl group A, which may be reinforced by the ferrocenylmethyl group.[80]

The authors concluded that the Ugi four-component reaction provides a powerful tool for integrating phenolic hydroxyl groups within a bis-amide molecule, giving substrates suitable for

exploring the mutual antioxidant effects among phenolic hydroxyl groups attached to different parts of the molecule.[80] It is actually found that the antioxidant property of the phenolic hydroxyl group at one tip of the molecule depends upon the isocyanide moiety at another tip of the molecule, in which ferrocenylmethyl isocyanide enhances the antioxidant effect markedly. Thus, the isocyanide moiety influences the antioxidant effect of the hydroxyl group at a long distance within a molecule. Mutual antioxidant effectiveness is also found among phenolic hydroxyl groups at the side chain.

Despite the wide range of combinations of hydroxyl bis-amides prepared by Wang and Liu, no derivatives with the *ortho*-catechol moiety as the amino acid side chain were report.

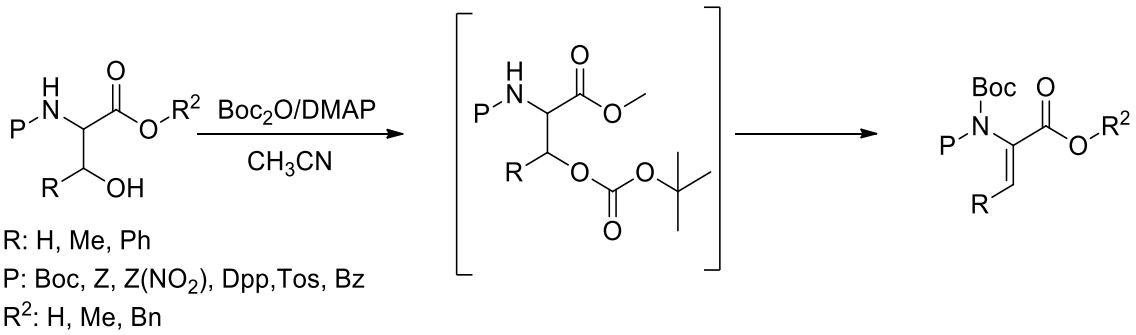
1.4.2. Synthesis of dehydroamino acids

Dehydroamino acids are not found free in nature, but have been synthesized as derivatives of amines, carboxylic esters or *N*-acylated derivatives.

The most important methods of synthesis of α,β-dehydroamino acids are those related to the biosynthetic pathway, that is by elimination reactions. There are several possible routes, such as: the dehydration of *N*-hydroxyamino acids; the dehydration of α-hydroxyamino acids; the direct oxidation of amino acids; or elimination reactions in β-hydroxy or β-mercapto amino acids.[81,82]

The simplest method for the synthesis of dehydroamino acids is by β-elimination reactions using as precursors β-hydroxyamino acids, such as serine and threonine, to give the corresponding dehydroamino acid, dehydroalanine (ΔAla) and dehydroaminobutyric acid (ΔAbu), respectively.[81,82] Various dehydration reagents have been used to carry out the β-elimination reactions.[83-87]

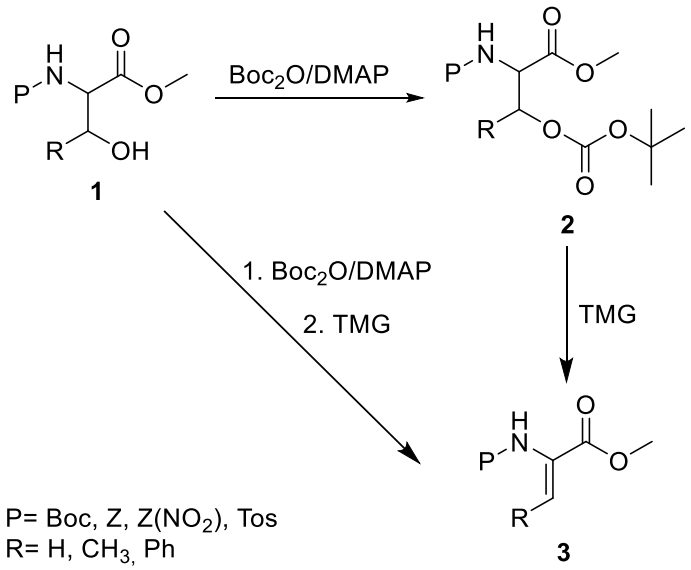
Ferreira *et al.*, developed an efficient method for the synthesis of *N,N*-diacyl-α,β-dehydroamino acid derivatives by using two equivalents of *tert*-butyldicarbonate (Boc_2O) and 4-dimethylaminopyridine (DMAP) as catalyst in dry acetonitrile. They found that formation of the *tert*-butyl carbonate derivative of the β-hydroxyamino acid occurred, followed by introduction of a Boc group as a second acylating group. The *tert*-butyl carbonate group is eliminated with formation of the corresponding dehydroamino acid derivative in excellent yield. This reaction is stereoselective, yielding the *Z*-isomers of dehydroaminobutyric acid and dehydrophenylalanine derivatives (Scheme 3).[88,89]



Scheme 3 - Synthesis of *N,N*-diacydehydroamino acids derivatives (adapted from references 88, 89).

This method was also applied in the preparation of dehydrodipeptides by reacting dipeptides containing serine, threonine or β-hydroxyphenylserine with three equivalents of *tert*-butyldicarbonate to give dehydrodipeptides in high yields (74-96%).

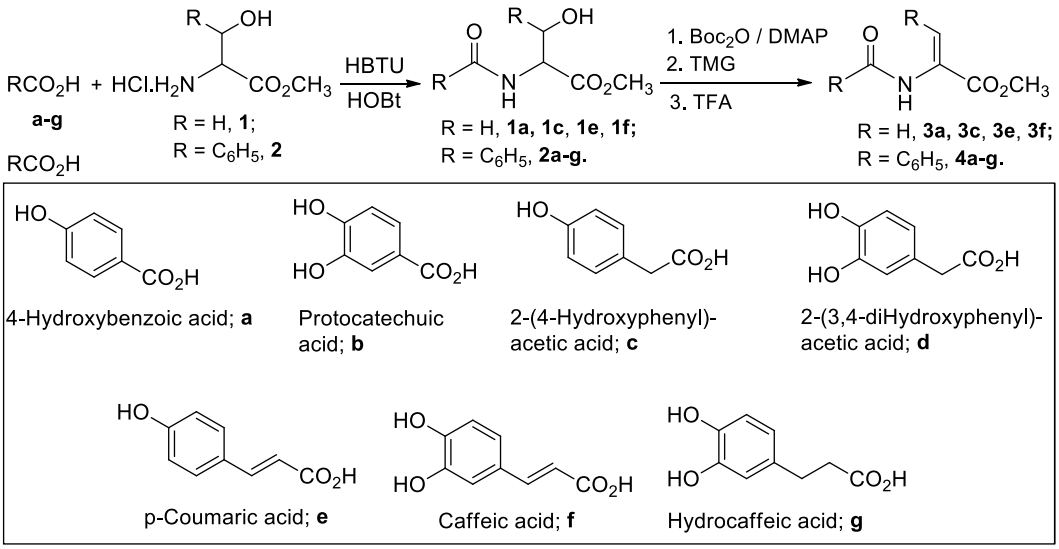
In order to allow the synthesis of *N*-monoprotected dehydroamino acid derivatives, a modification of this method was subsequently reported.[90] Thus, by reacting β-hydroxyamino acid derivatives with one equivalent of Boc_2O and DMAP it was possible to obtain the corresponding β-carbonates that undergo β-elimination by treatment with *N,N,N',N'*-tetramethylguanidine (TMG) (Scheme 4).



Scheme 4 – Synthesis of *N*-acyldehydroamino acids derivatives (adaptaded from reference 90).

1.4.2.1. Synthesis of phenolic acids coupled with dehydroamino acids

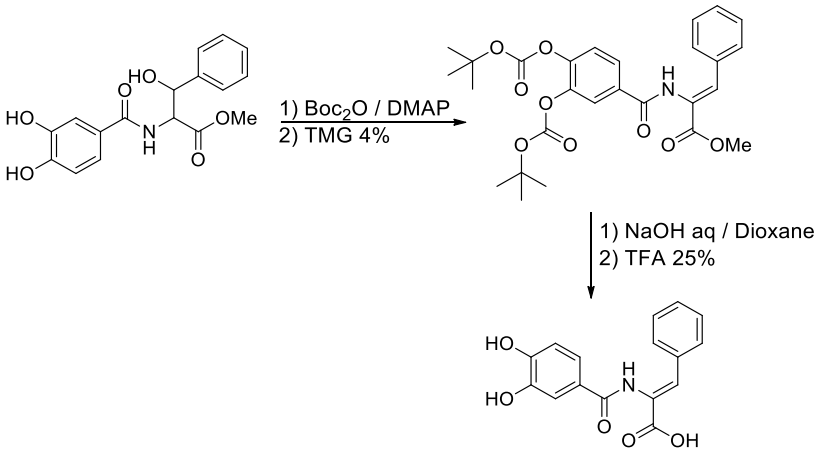
Monteiro *et al.*[64] developed an innovative strategy for the synthesis of the methyl esters of *N*-phenoyl and *N*-catechoyl dehydroalanine and dehydrophenylalanines. *N*-Protected β-hydroxyamino acid derivatives were treated sequentially in a one-pot procedure by reaction with *tert*-butyldicarbonate and dimethylaminopyridine, followed by treatment with *N,N,N',N'*-tetramethylguanidine and cleavage of the aromatic *O-tert*-butyloxycarbonyl groups with trifluoroacetic acid to give methyl esters of *N*-phenolic or *N*-catecholic dehydroalanine or dehydrophenylalanine (Scheme 5, compounds **3a**, **3c**, **3e**, **3f** and **4a-4g**).



Scheme 5 – Synthesis of the methyl esters of *N*-phenoyl and *N*-catechoyl dehydroalanine and dehydrophenylalanine (adapted from reference 64).

Due to the high hydrophilic character of compounds **1b**, **1d** and **1g** when compared with the other serine derivatives (compounds **1a**, **1c**, **1e** and **1f**) the former could not be isolated. The lower hidrophylicity of the phenylserine derivatives obtained allowed the preparation of all *N*-protected phenylserine derivatives in good yields (compounds **2a-2g**).

Due to the relative ease with which catechol groups oxidize, are prone to nucleophilic attack and phenolic coupling reactions in basic media, it is not possible to remove the methyl esters from compounds such as **3f** or **4f**. Alternatively, the *C*-deprotected *N*-catechoyl dehydroamino acid derivatives were prepared by a sequential *tert*-butyloxycarbonylation and dehydration; followed by treatment with base to remove the methyl ester and cleavage of the Boc groups with TFA.



Scheme 6 - Two-step procedure to prepare *C*-deprotected *N*-protocatechoyl dehydrophenylalanine (adapted from reference 64).

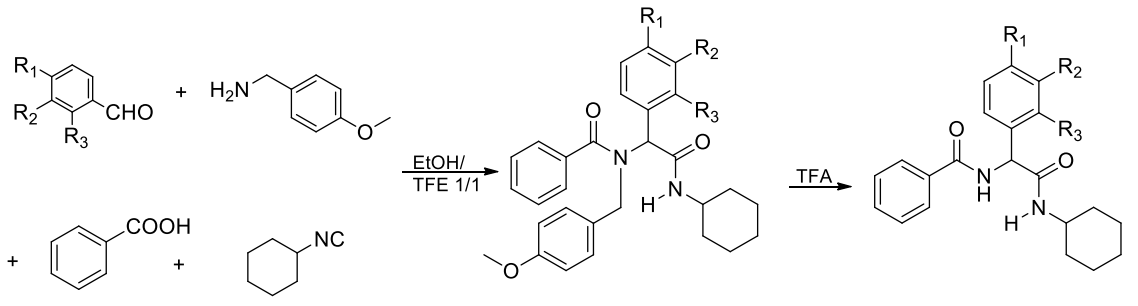
2. Results and discussion

2.1. Synthesis of phenolic and catecholic amino acid derivatives by the modified multicomponent Ugi reaction

To obtain new phenolic and catecholic amino acids, a strategy based on modified Ugi multicomponent reaction was employed. Multicomponent reactions (MCR) are one-pot reactions that form products from three or more different starting compounds.[65] The reagents may be different individuals molecules or they may be different functional groups in the same reagent.

The Ugi reaction is the condensation of an aldehyde, an amine, a carboxylic acid and an isocyanide in one-step.[91] This reaction has enabled the preparation of molecules with high diversity and is a valuable tool for generating α-amino acid derivatives. The combination of various carboxylic acids, aldehydes, amines or isocyanides gives hundreds of bis-amides with different biological activities. The Ugi four-component reaction has allowed the integration of phenolic hydroxyl groups within a bis-amide molecule, giving substrates suitable for exploring the antioxidant effects of different phenolic components.[80]

The procedure proposed uses as amine component 4-methoxybenzylamine and as isocyanide fragment cyclohexyl isocyanide. The carboxylic acid component used initially was benzoic acid and the aldehyde component bared the phenolic or catechol moiety (Scheme 7). The Ugi adduct formed would be subsequently treated with trifluoroacetic acid (TFA) to remove the methoxybenzylamine group and eventually also the cyclohexylamine.



R_1 = H, OH; R_2 = H, OCH_3,OH; R_3=H, OH.

Scheme 7 – Synthesis of phenolic and catecholic amino acid derivatives by the modified multicomponent Ugi reaction.

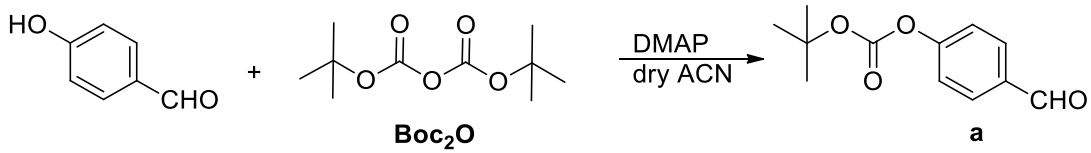
2.1.1. Synthesis of *O-tert*-butyloxycarbonylated benzaldehydes

Use of phenolic or catecholic aldehyde components in the Ugi reaction requires previous protection of the hydroxyl functions. This need arises from the limitations of the use of *ortho*-

catechol moiety in synthesis due to its high instability in basic environment. This basic environment, in this case, arises from the nature of the amine component.

The choice of hydroxyl protection was as *tert*-butyl carbonates, which has been recently applied in the synthesis of phenolic and catecholic dehydroamino acid derivatives. The advantage of this type of protection is that *O*-*tert*-butyloxycarbonylated benzaldehydes can be easily obtained by reaction of hydroxybenzaldehydes with *tert*-butyldicarbonate in the presence of dimethylaminopyridine (DMAP) as catalyst.[64] Furthermore, this temporary protection can be removed in the subsequent treatment with TFA.

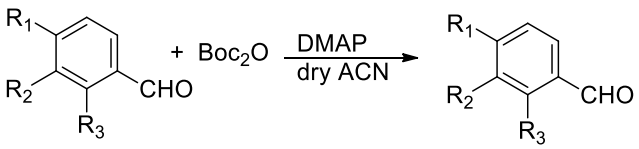
In order to test the *O*-*tert*-butyloxycarbonylation of hydroxybenzaldehydes, the simpler aldehyde, 4-hydroxybenzaldehyde, was used. To this benzaldehyde, one equivalent of *tert*-butyldicarbonate (Boc_2O) was added followed by 0.1 equivalents of dimethylaminopyridine (DMAP) in dry acetonitrile (Scheme 8). After reacting for 4h, treatment of the reaction mixture gave *tert*-butyl (4-formylphenyl) carbonate (compound **a**, Scheme 8)[92] in 96% yield.



Boc_2O **a**

Scheme 8 – Synthesis of *tert*-butyl (4-formylphenyl) carbonate.

The high yield obtained in this reaction, allowed us to proceed with the protection of different aldehydes. Thus, three more benzaldehydes bearing hydroxyl functions, namely, 3-methoxy-4-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde and 2,3-dihydroxybenzaldehyde were reacted with *tert*-butylpyrocarbonate in the presence of dimethylaminopyridine to give *O*-(*tert*-butyloxycarbonyl) benzaldehydes (Scheme 9, compounds **b**[93], **c**[94] and **d**). All compounds including the highly sterically hindered di-*tert*-butyl (3-formyl-1,2-phenylene) dicarbonate, compound **d**, could be obtained in excellent yields (Table 3).



R_1=OH, R_2, R_3=H;
R_1=OH, R_2=OCH_3, R_3=H;
R_1, R_2=OH, R_3=H;
R_1=H, R_2,R_3=OH.

R_1=OBoc, R_2, R_3=H, **a**;
R_1=OBoc, R_2=OCH_3, R_3=H, **b**;
R_1, R_2=OBoc, R_3=H, **c**;
R_1=H, R_2,R_3=OBoc, **d**.

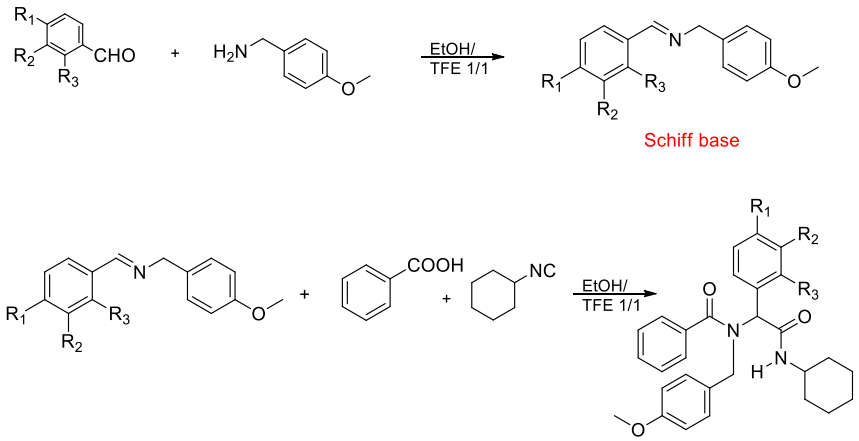
Scheme 9 – Synthesis of *O*-(*tert*-butyloxycarbonyl) benzaldehydes.

Table 3 – Yields and characteristics of *O*-(*tert*-butyloxycarbonyl) benzaldehydes.

Compound	IUPAC Name	Yield	Characteristics
a	*tert*-Butyl (4-formylphenyl) carbonate	96%	white solid
b	*tert*-Butyl (4-formyl-2-methoxyphenyl) carbonate	95%	white solid
c	di-*tert*-Butyl (4-formyl-1,2-phenylene) dicarbonate	94%	light brown oil
d	di-*tert*-Butyl (3-formyl-1,2-phenylene) dicarbonate	96%	light brown solid

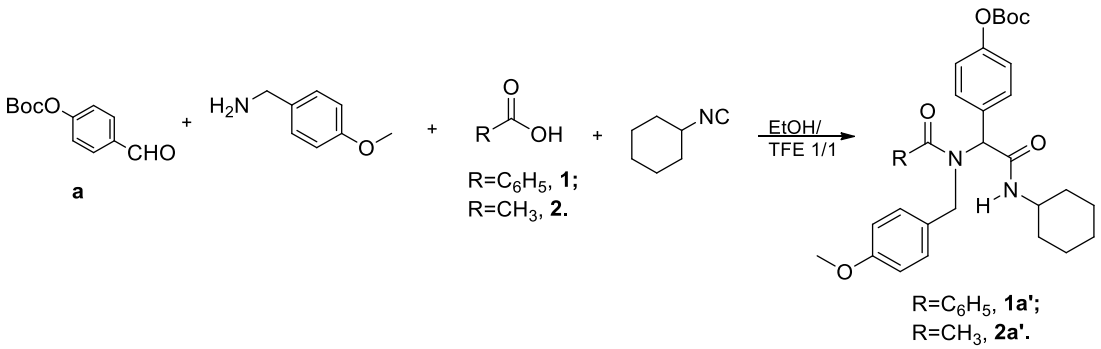
2.1.2 Synthesis of Ugi adducts

The Ugi reaction is usually carried out in two steps. Initially an imine is obtained by condensation of an amine with an aldehyde to give the corresponding Schiff base (Scheme 10). Subsequently, the carboxylic acid and the isocyanide components are added to give the classical Ugi skeleton. Carrying out the reaction in these two steps has proved to increase the overall yield in Ugi adduct.



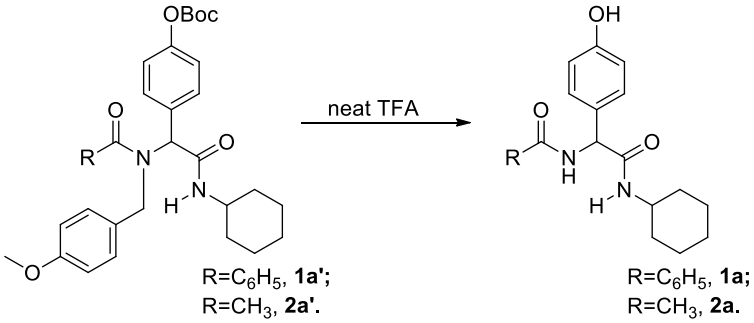
Scheme 10 – The Ugi reaction in two steps (adapted from reference 91).

A preliminary test was carried out using as reactants *tert*-butyl (4-formylphenyl) carbonate, 4-methoxybenzylamine, benzoic acid and cyclohexyl isocyanide. To a solution of *tert*-butyl (4-formylphenyl) carbonate in ethanol/trifluorethanol (TFE) 1/1 under a stream of nitrogen, 4-methoxybenzylamine was added. After 4 hours the carboxylic acid and cyclohexyl isocyanide were added. The reaction was stirred at room temperature for 48 h, after which, treatment of the reaction mixture gave Ugi adduct **1a'** as a white solid in 94% yield (Scheme 11). The proton NMR spectrum of the compound gave the expected peaks with the diastereotopic CH_2 protons of the benzylamine group appearing as two signals at 4.41 ppm and 4.63 ppm.



Scheme 11 – Synthesis of the cyclohexylamides of *N*-benzoyl and *N*-acetyl, *N*-(4-metoxybenzylamino)-[4-(*O-tert*-butyloxycarbonyl)phenyl]-glycine (compounds **1a'** and **2a'**).

The Ugi adduct was treated with TFA to give compound **1a** as a white solid with 34% yield (Scheme 12). The proton NMR spectrum did not exhibit the peaks corresponding to the 4-methoxybenzyl group, previously cited, neither the peaks corresponding to the Boc group that typically appear as a singlet around 1.5 ppm. However, peaks corresponding to the cyclohexylamide were still present. Thus, removal of the hydroxyl protecting group *tert*-butyloxycarbonyl (Boc) and of the 4-methoxybenzyl group occurred; however, the cyclohexylamide was maintained.



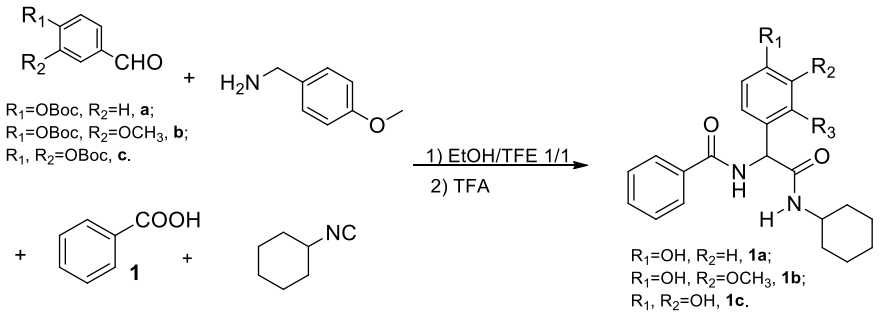
Scheme 12 – Synthesis of the cyclohexylamides of *N*-benzoyl and *N*-acetyl, 4-(hydroxyphenyl)-glycine (compounds **1a** and **2a**).

To verify if the bulkiness of the aromatic ring of benzoic acid was responsible for lack of cleavage of the cyclohexylamide, the Ugi reaction was performed with acetic acid to give **2a'** in quantitative yield as a light yellow oil (Scheme 12). Treatment of compound **2a'** with TFA, again resulted in removal of the Boc and the 4-methoxybenzyl groups, without cleavage of the cyclohexylamine (compound **2a**, Scheme 12). Thus, treatment of Ugi adducts **1a'** and **2a'** with TFA allowed the simultaneous removal of the *tert*-butyloxycarbonyl and the methoxybenzyl groups, without affecting the cyclohexylamide. This is in agreement with previous results by Maia *et al.* in the synthesis of

N-acyl-α,α-dialkylglycines by cleavage of Ugi adducts with neat TFA.[95] Treatment in these conditions of *N*-benzoyl-*N,α,α*-trialkylglycine amides formed by reaction of 4-methoxybenzylamine, benzoic acid, cyclohexyl isocyanide and the more bulky dibenzylketone, resulted in removal of the methoxybenzyl group without amide cleavage. The authors reported that in neat TFA the *N*-alkyl group of the bulkier *N*-benzoyl, *N*-(4-methoxybenzyl) *α,α*-dibenzylglycine derivatives cleaves faster than their amide bond. They concluded that, for acidolytic cleavage of the C-terminal amide to occur, the presence of an alkyl/aryl substituent at the amino acid nitrogen atom is required. Thus, the phenyl group directly linked to the α–carbon in adducts **1a'** and **2a'** seems to exert a steric effect comparable to that reported for the α,α-dibenzyl moiety in *N*-acyl-*N,α,α*-trialkylglycine amides.

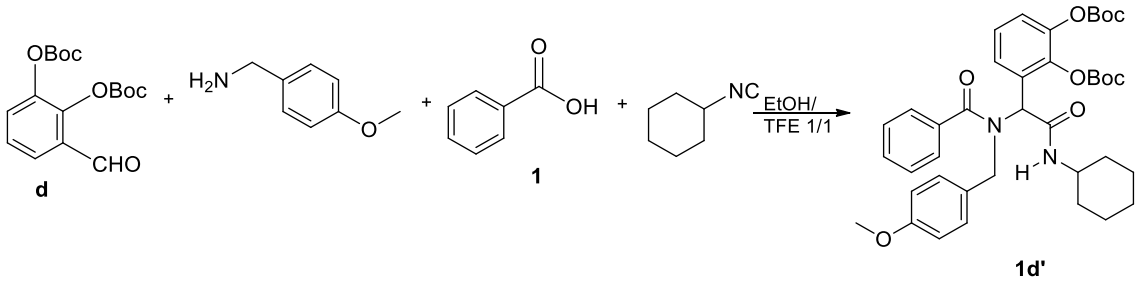
Compound **1a** could also be obtained in a one-pot procedure consisting of the condensation reaction, followed by evaporation of the solvent at reduced pressure and treatment of the residue with TFA (Scheme 13). Compound **1a** was obtained in the one-pot procedure in 63% yield, while the overall yield of the two steps reaction was 34%.

tert-Butyl (4-formyl-2-methoxyphenyl) carbonate and di-*tert*-butyl (4-formyl-1,2-phenylene) dicarbonate (Scheme 13, compounds **b** and **c**, respectively) were reacted in the same conditions to give *N*-benzoyl, 4-hydroxy-3-methoxy and 3,4-dihydroxy phenylglycine cyclohexylamides in 76% and 66% yields, respectively (compounds **1b** and **1c**, Scheme 13).



Scheme 13 – One-pot synthesis of the cyclohexylamides of *N*-benzoyl, (hydroxyphenyl)glycines (compounds **1a**, **1b** and **1c**).

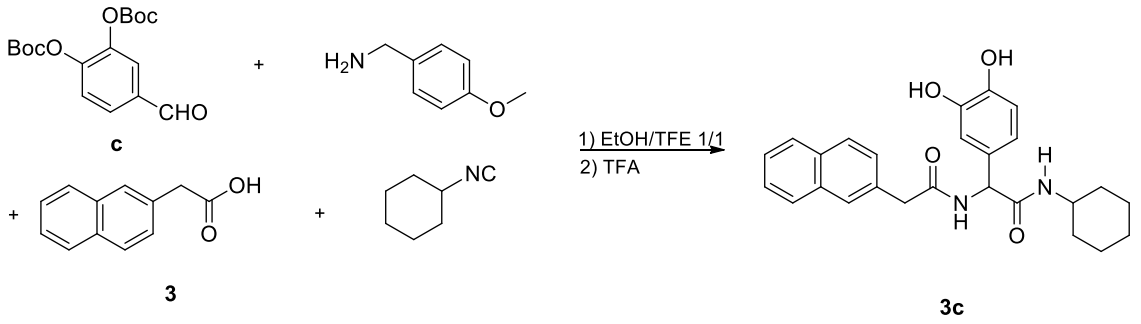
An attempt to carry out the condensation reaction using di-*tert*-butyl (3-formyl-1,2-phenylene) dicarbonate (compound **d**, Scheme 14) and benzoic acid gave only trace amounts of the corresponding Ugi adduct (compound **1d'**, Scheme 14).



Scheme 14 – Synthesis of the cyclohexylamide of *N*-benzoyl *N*-(4-metoxybenzylamino)-[2,3-(*O*-di-*tert*-butyloxycarbonyl)phenyl]glycine (compound **1d'**).

This can be explained by the bulky Boc group in position 2, which causes high steric hindrance. Wang and Liu in a similar study, also obtained a low yield in Ugi adduct when they placed a bulky group at position 2 of the benzaldehyde. In this case, the yield in Ugi adduct was only 51%, which was significantly lower than the same reaction with an aldehyde with a hydrogen atom at position 2, for which they obtained 83% yield.[80] Due to the low yield in compound **1d'**, attempts at treatment with TFA was not carried out.

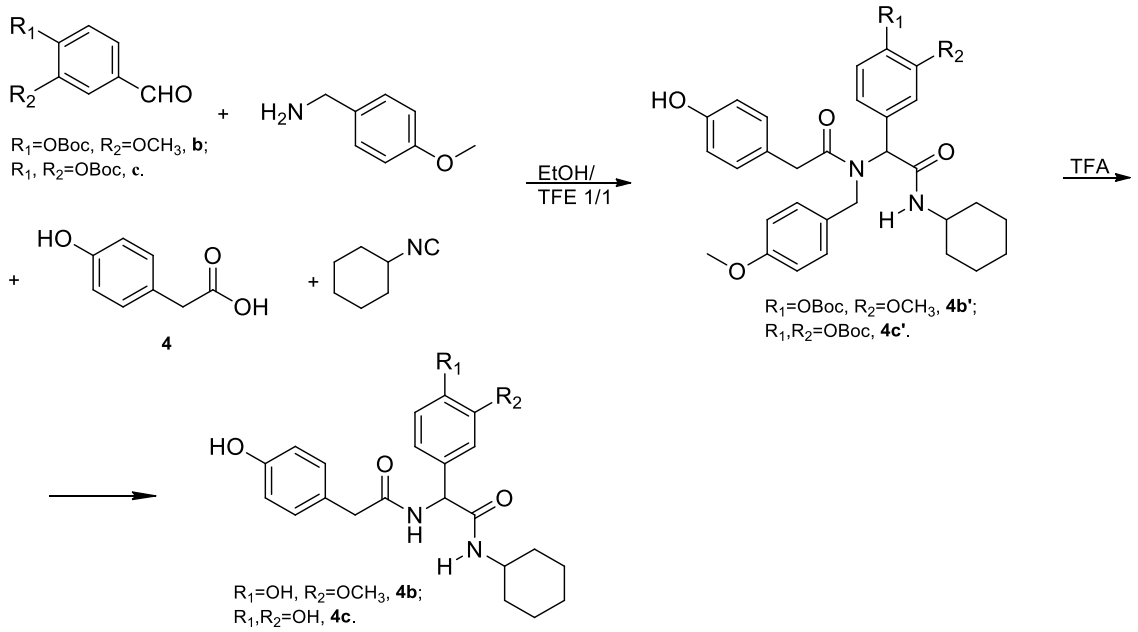
The one-pot reaction was carried out substituting 2-naphthylacetic acid for benzoic acid and using di-*tert*-butyl (4-formyl-1,2-phenylene) dicarbonate to give the *N*-(2-naphthyl)acetyl, (3,4-dihydroxyphenyl)glycine amide in 78% yield (compound **3c**, Scheme 15).



Scheme 15 – One-pot synthesis of the cyclohexylamide of *N*-(2-naphthyl)acetyl (3,4-dihydroxyphenyl)glycine (compound **3c**).

After obtaining good results in the syntheses carried out with carboxylic acids with only aromatic rings or a methyl group, it was decided to use carboxylic acids with a hydroxyl group, namely 4-hydroxyphenylacetic acid and *p*-coumaric acid. Thus, to a solution of *tert*-butyl (4-formyl-2-methoxyphenyl) carbonate in ethanol/2,2,2-trifluoroethanol, 4-methoxybenzylamine was added. After 4 hours, 4-hydroxyphenylacetic acid and cyclohexyl isocyanide were added and left to react to give the corresponding Ugi adduct **4b'** (Scheme 16) as a white solid with 97% yield. Then

trifluoroacetic acid was added to the adduct to give compound **4b** (Scheme 16) as a light pale solid in 59% yield.



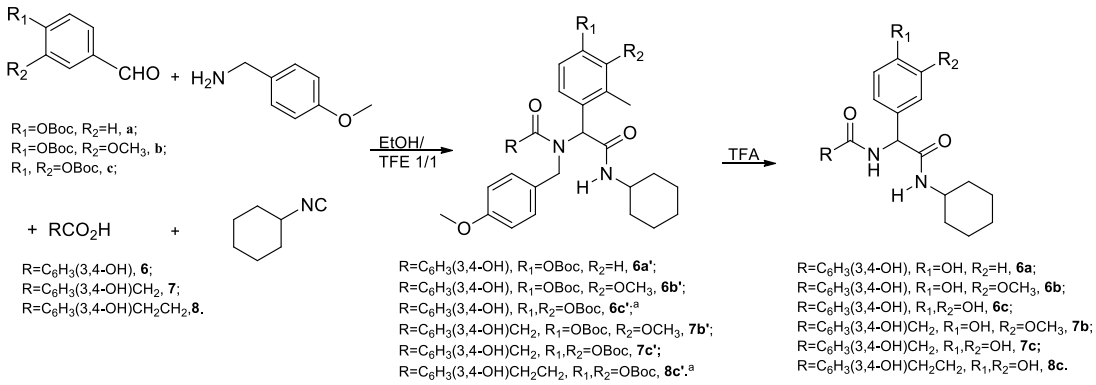
Scheme 16 – Synthesis of the cyclohexylamides of *N*-(4-hydroxyphenyl)acetyl, (4-hydroxy, 3-methoxyphenyl)glycine and (3,4-dihydroxyphenyl)glycine (compound **4b** and **4c**).

The same procedure was carried out using the di-*tert*-butyl (4-formyl-1,2-phenylene) dicarbonate and 4-hydroxyphenylacetic acid to give compound **4c'** (Scheme 16) as a light brown oil with 92% yield. Cleavage with TFA gave compound **4c** as a light orange oil with 80% yield. All the structures of these compounds were confirmed using ^{1}H and ^{13}C NMR spectroscopy.

The same procedure was carried out using *tert*-butyl (4-formyl-2-methoxyphenyl) carbonate and *p*-coumaric acid to give the corresponding Ugi adduct **5b'** as a white solid with 83% yield. Treatment of the Ugi adduct with trifluoroacetic acid failed to give compound **5b**.

The results obtained led us to explore the possibility of adding a second catecholic function to the amino acid derivatives by using as carboxylic acid component a catecholic acid. These cathecolic acids were tested without hydroxyl protection, since when the acid component is added, all 4-methoxybenzylamine has been consumed. Thus, *tert*-butyl (4-formylphenyl) carbonate was reacted in the same conditions, substituting protocatechuic acid for benzoic acid to give the Ugi adduct **6a'** in quantitative yield (Scheme 17). Treatment of compound **6a'** with TFA gave the *N*-protocatechoyl (4-hydroxyphenyl)glycine derivative in 72% yield (compound **6a**, Scheme 17). By reacting *tert*-butyl (4-formyl-2-methoxyphenyl) carbonate or di-*tert*-butyl (4-formyl-1,2-phenylene)

dicarbonate with protocatechuic acid the corresponding *N*-protocatechoyl, (hydroxyphenyl)glycine amides were obtained in good yields (compounds **6b** and **6c**, respectively, Scheme 17).

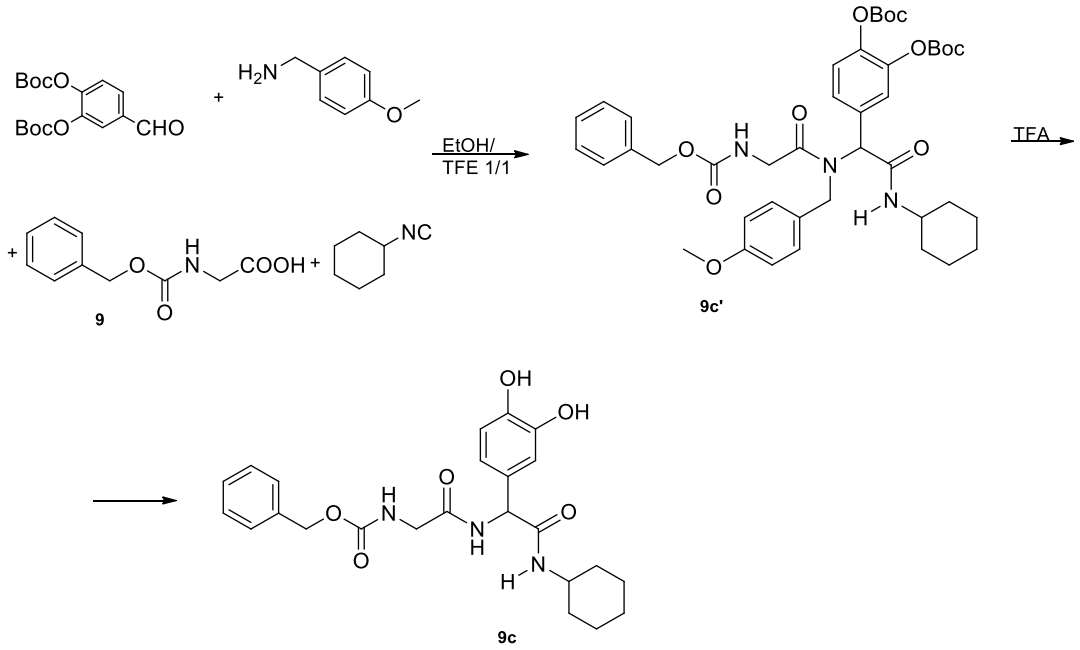


Scheme 17 – Synthesis of the cyclohexylamides of *N*-protocatechoyl, *N*-(3,4-dihydroxyphenyl)acetyl and *N*-hydrocaffeoyl, (4-hydroxy, 3-methoxyphenyl)glycine and (3,4-dihydroxyphenyl)glycine (compound **6a**, **6b**, **6c**, **7b**, **7c** and **8c**).

The same procedure using 3,4-dihydroxyphenylacetic acid and *tert*-butyl (4-formyl-2-methoxyphenyl) carbonate gave the corresponding Ugi adduct **7b'** as a white solid with 93% yield. Treatment of **7b'** with trifluoroacetic acid gave compound **7b** as a white solid in 44% yield. Di-*tert*-butyl (4-formyl-1,2-phenylene) dicarbonate was reacted in the same conditions to give the *N*-(3,4-dihydroxyphenyl)acetyl, (3,4-dihydroxyphenyl)glycine derivative in 63% yield (compound **7c,** Scheme 17).

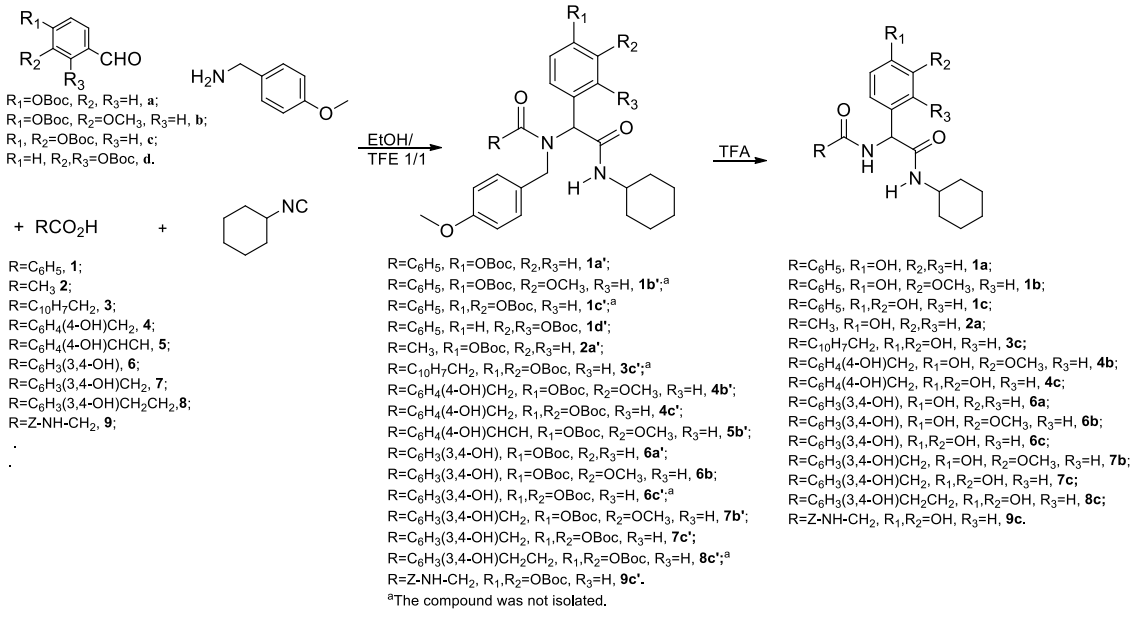
The one-pot reaction was carried out with hydrocaffeic acid and using di-*tert*-butyl (4-formyl-1,2-phenylene) dicarbonate to give the *N*-hydrocaffeoyl (3,4-dihydroxyphenyl)glycine cyclohexylamide in 74% yield (compound **8c**, Scheme 17).

In order to test the possibility of obtaining a dipeptide derivative baring a (hydroxyphenyl)glycine residue, the reaction was carried out using as acid component *N*-benzyloxycarbonylglycine. Thus, reaction in the same conditions using di-*tert*-butyl (4-formyl-1,2-phenylene) dicarbonate and *N*-benzyloxycarbonylglycine gave compound **9c'** as a light pink solid in 94% yield. Cleavage with TFA gave the cyclohexylamide of *N*-benzyloxycarbonylglycyl, (3,4-dihydroxyphenyl)glycine as a white solid in 74% yield (compound **9c**, Scheme 18).



Scheme 18 – Synthesis of cyclohexylamide of *N*-benzyloxycarbonylglycyl, (3,4-dihydroxyphenyl)glycine (compound **9c**).

Thus, a wide range of *N*-acyl (hydroxyphenyl)glycine amides could be obtained in good yields by reacting hydroxybenzaldehyde derivatives with 4-methoxybenzylamine, cyclohexyl isocyanide and benzoic acid, phenolic acids or catecholic acids to give Ugi adducts that were subsequently treated with trifluoroacetic acid (Scheme 19, Table 4).



Scheme 19 – Synthesis of *N*-acyl (hydroxyphenyl)glycine amides.

Table 4 – Yields obtained in the synthesis of phenolic and catecholic Ugi adducts and in their treatment with trifluoroacetic acid to give *N*-acyl, (hydroxyphenyl)glycine amides.

O-(*tert*-Butyloxycarbonyl) benzaldehyde	Carboxylic acid	Ugi adduct	Yield (%)	Cyclohexylamide of *N*-acyl, (hydroxyphenyl)glycine	Yield (%)
tert-Butyl (4-formylphenyl) carbonate, **a**	Benzoic acid, **1**	**1a'**	94	**1a**	34
tert-Butyl (4-formyl-2-methoxyphenyl) carbonate, **b**	Benzoic acid, **1**	**1b'**	-	**1b**	76
di-*tert*-Butyl (4-formyl-1,2-phenylene) dicarbonate, **c**	Benzoic acid, **1**	**1c'**	-	**1c**	66
di-*tert*-Butyl (3-formyl-1,2-phenylene) dicarbonate, **d**	Benzoic acid, **1**	**1d'**	17	**1d**	-
tert-Butyl (4-formylphenyl) carbonate, **a**	Acetic acid, **2**	**2a'**	Quantitative	**2a**	-
di-*tert*-Butyl (4-formyl-1,2-phenylene) dicarbonate, **c**	2-Naphtylacetic acid, **3**	**3c'**	-	**3c**	78
tert-Butyl (4-formyl-2-methoxyphenyl) carbonate, **b**	4-Hydroxyphenylacetic acid, **4**	**4b'**	97	**4b**	59
di-*tert*-Butyl (4-formyl-1,2-phenylene) dicarbonate, **c**	4-Hydroxyphenylacetic acid, **4**	**4c'**	92	**4c**	80
tert-Butyl (4-formyl-2-methoxyphenyl) carbonate, **b**	*p*-Coumaric acid, **5**	**5b'**	83	**5b**	-
tert-Butyl (4-formylphenyl) carbonate, **a**	Protocatechuic acid, **6**	**6a'**	Quantitative	**6a**	72
tert-Butyl (4-formyl-2-methoxyphenyl) carbonate, **b**	Protocatechuic acid, **6**	**6b'**	Quantitative	**6b**	68
di-*tert*-Butyl (4-formyl-1,2-phenylene) dicarbonate, **c**	Protocatechuic acid, **6**	**6c'**	-	**6c**	69
tert-Butyl (4-formyl-2-methoxyphenyl) carbonate, **b**	3,4-diHydroxyphenylacetic acid, **7**	**7b'**	93	**7b**	44
di-*tert*-Butyl (4-formyl-1,2-phenylene) dicarbonate, **c**	3,4-diHydroxyphenylacetic acid, **7**	**7c'**	86	**7c**	63
di-*tert*-Butyl (4-formyl-1,2-phenylene) dicarbonate, **c**	Hydrocaffeic acid, **8**	**8c'**	-	**8c**	74
di-*tert*-Butyl (4-formyl-1,2-phenylene) dicarbonate, **c**	*N*-Benzyloxycarbonylglycine acid, **9**	**9c'**	94	**9c**	74

2.2. DPPH Radical Essay

The DPPH radical assay method is one of most commonly used to evaluate the antiradicalar ability of compounds and measures their reactivity with the stable free radical, 2,2-diphenyl-1-picrylhydrazyl (Figure 12).

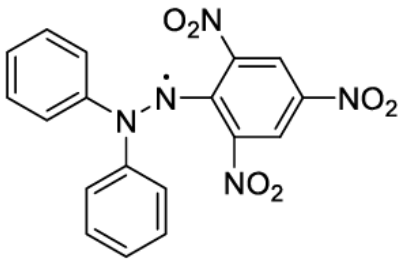


Figure 12 – Chemical structure of 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH·).

The DPPH• radical exhibits a maximum absorption at 515 nm (methanolic solutions of the radical have a strong violet colour). However, after reduction by an antioxidant or a radical species (R·), a decrease in the absorbance at that wavelength is observed. When the unpaired electron of the DPPH· nitrogen atom receives a hydrogen atom from the antioxidant compound, colour change occurs (Figure 13).[60]

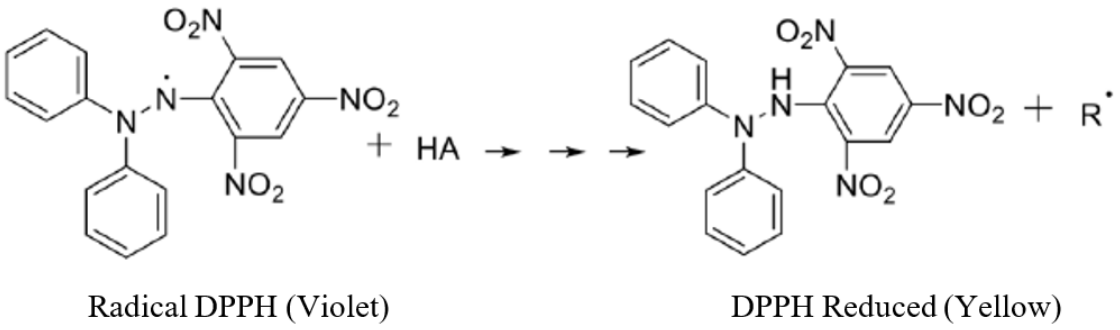


Figure 13 – Mechanism of DPPH reaction with antioxidant.[60]

Determination of the radical-scavenging activity of the *N*-acyl (hydroxyphenyl)glycines was carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH•) as a stable radical.[96] For each compound the EC_{50} values (relative concentration of antioxidant required to lower the initial DPPH• concentration by 50%) were determined after several reaction times and compared to the values obtained for protocatechuic acid.

As expected, the radical-scavenging activity of the monophenol derivative **1a** was very weak. However, a significant increase in activity occurs when a methoxy group is added in *ortho* position to the hydroxyl group (compound **1b**) (a six-fold increase for EC_{50} determined after 5 minutes). The substitution of this methoxy group by another hydroxyl group (compound **1c**), increases more than ten times the radical-scavenging activity. A slightly higher activity is observed when (2-naphthyl)acetyl is substituted for benzoyl as *N*-acyl group (compound **3c**). The *N*-protocatechoyl 4-hydroxylphenylglycine derivative (compound **6a**) showed radical-scavenging activity comparable to

that of compound **1c**, which also has an *ortho*-dihydroxyaryl function as amino acid side chain. Introduction of a methoxy group in *ortho* position to the hydroxyl group (compound **6b**) causes a 25% increases in activity as determined after 5 minutes. Substitution of the methoxy group with a second hydroxyl group (compound **6c**) causes a further 30% increase in activity. The *N*-hydrocaffeoyl 3,4-dihydroxyphenylglycine derivative (compound **8c**) showed similar activity to the *N*-protocatechoyl derivative **6c**. The radical-scavenging activities of compounds **6c** and **8c** were almost double the activities of derivatives with a single catecholic group (compounds **1c** or **6a**).

These results show a direct correlation between radical-scavenging capacity against the hindered DPPH radical and the number of hydroxyl or catechol groups present in the glycine derivatives. A high increase in activity with the increase in number of hydroxyl or catechol groups is observed with the monocatecholic derivatives (compounds **1c** and **6a**) having twice the radical-scavenging activity of protocatechuic acid, while the *N*-catechoyl derivatives **6c** and **8c** have almost four times this activity. Thus, the introduction of the catecholic moiety in an amino acid structure induces a significant increase in its intrinsic radical scavenging activity.

2.3. Synthesis of phenolic and catecholic derivatives of dehydrophenylalanine

Amino acids coupled with phenolic acids and/or phenolic amines can be obtained from natural sources such as fruits, vegetables, and beverages, or synthetically. It is assumed that these bioactive substances are involved in suppression of deleterious effects of oxidative stress and have a wide range of biological activities such as as antioxidant,[3,47-50] anticancer[4] and antimicrobial.[5, 51-55]

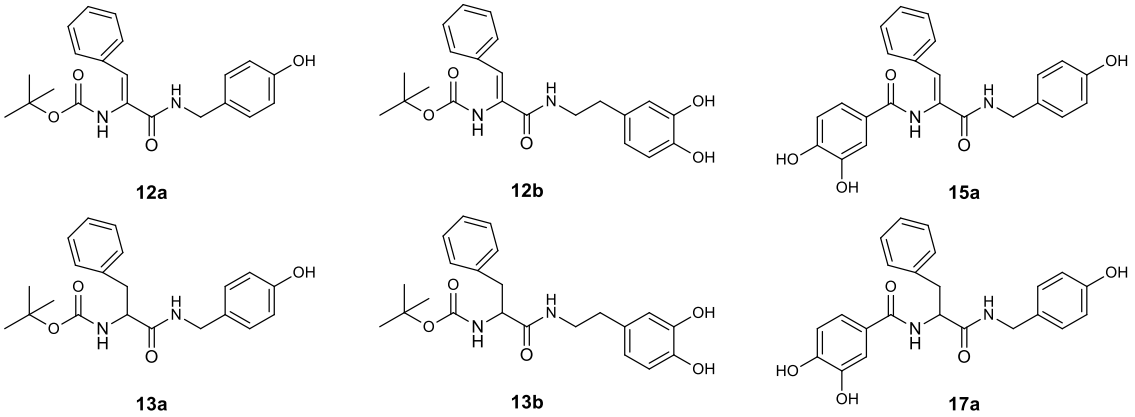
Studies have confirmed that molecular combinations improve the antioxidant efficiency of natural antioxidants and can be useful to investigate structure activity relationships. For example, phenolic acid-amino acid and phenolic acid-amino acid-dopamine conjugates have shown to improve the antioxidant activity of natural amino acids due to the presence of phenolic functions.[46]

Dehydroamino acids constitute an important class of non-proteinogenic amino acids with various biological activities, including antioxidant.[29] A strategy in which dehydroamino acids are coupled with phenolic or catecholic acids and/or phenolic or catecholic amines could yield compounds with enhanced antioxidant activities.

An efficient method for the synthesis of dehydroamino acids was developed by Ferreira *et al.* and has been used to prepare a wide range of dehydroamino acid derivatives.[88-90] Herein, we propose to explore this method in order to obtain combinations of a dehydroamino acid with phenolic and catecholic amines and with phenolic acids. Thus, we proposed the synthesis of

phenoyl amides of *N*-phenoyl dehydrophenylalanine and to compare their antioxidant activities with the corresponding derivatives of the saturated amino acid phenylalanine.

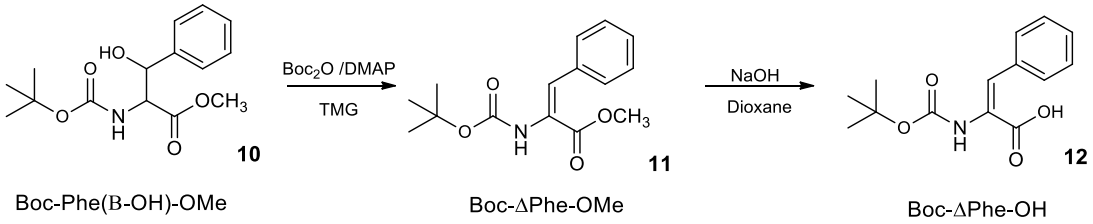
The following combinations of *N*-acyl dehydrophenylalanine and phenyalanine amides were prepared:



2.3.1 Synthesis of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine

In order to obtain an *N*-acyl dehydrophenyalanine derivative that could be coupled with phenolic or catecholic amines, *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine was prepared.

The methyl ester of *N*-(*tert*-butyloxycarbonyl) phenylserine was reacted with *tert*-butyldicarbonate (Boc_2O) and 4-dimethylaminopyridine (DMAP) as catalyst, followed by treatment with *N,N,N',N'*-tetramethylguanidine (TMG).[90] Thus, to a solution of the methyl ester of *N*-(*tert*-butyloxycarbonyl) phenylserine in dry acetonitrile, 0.11 equivalent of DMAP were added, followed by 1.1 equivalents of Boc_2O under rapid stirring at room temperature. After stirring for 16 hours the reaction mixture was treated with 2% in volume of *N,N,N',N'*-tetramethylguanidine (TMG) to give the methyl ester of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine (compound **11**, Scheme 20) as a white solid in 98% yield.

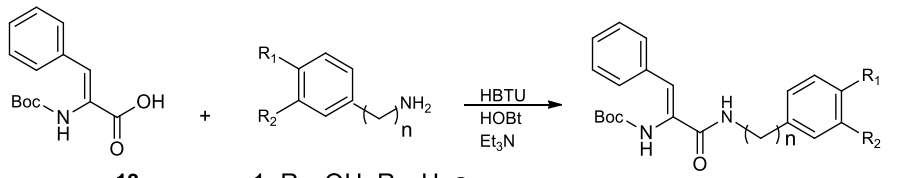


Scheme 20 – Synthesis of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine.

Removal of the methyl ester was carried out by treating compound **11** in dioxane/ NaOH (1 mol.dm^{-3}) under rapid stirring at room temperature, to give *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine (compound **12**, Scheme 20) as a white solid in 96% yield.

2.3.2. Synthesis of the phenoyl amides of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine and phenylalanine

Coupling of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine with phenoyl amines was carried out using HOBt/HBTU as coupling agents. Thus, compound **12** was dissolved in acetonitrile and 1.0 equivalent of HOBt added, followed by addition of 1.0 equivalent of HBTU and 1.0 equivalent of 4-hydroxybenzylamine. After reacting for 18 h treatment of the reaction mixture gave the 4-hydroxybenzylamide of *N*-(*tert*-butyloxycarbonyl) dehydrophenyalanine (compound **12a**, scheme 21) as a yellow oil in 88% yield.



12

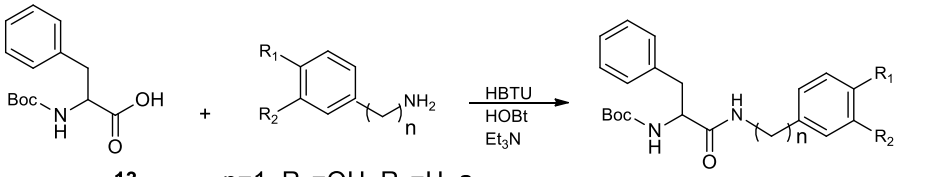
n=1, R_1=OH, R_2=H, **a**
n=2, R_1, R_2=OH, **b**

n=1, R_1=OH, R_2=H, **12a**
n=2, R_1, R_2=OH, **12b**

Scheme 21 - Synthesis of the 4-hydroxybenzylamide and the dopamide of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine.

The same procedure using dopamine hydrochloride gave the dopamide of *N*-(*tert*-butyloxycarbonyl) dehydrophenyalanine (compound **12b**, Scheme 21) as a light yellow solid in 97% yield.

In order to obtain the saturated references, *N*-(*tert*-butyloxycarbonyl) phenylalanine was reacted with 4-hydroxybenzylamine and dopamine in the same conditions, giving compounds **13a** and **13b** in 94% and 91% yield, respectively (Scheme 22).



13

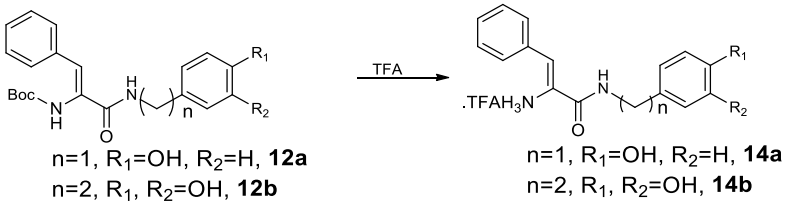
n=1, R_1=OH, R_2=H, **a**
n=2, R_1, R_2=OH, **b**

n=1, R_1=OH, R_2=H, **13a**
n=2, R_1, R_2=OH, **13b**

Scheme 22 - Synthesis of the 4-hydroxybenzylamide and the dopamide of *N*-(*tert*-butyloxycarbonyl) phenylalanine.

2.3.3. Synthesis of a phenoyl amide of *N*-protocatechoyl dehydrophenylalanine and phenylalanine

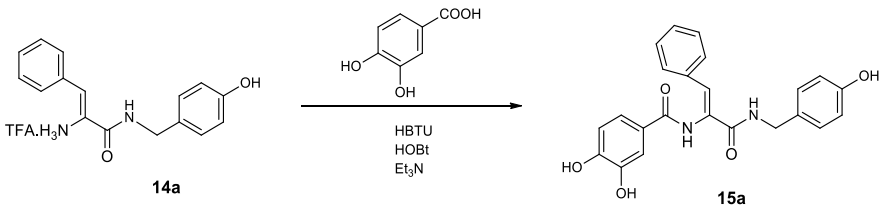
In order to conjugate the phenoyl amides of dehydrophenylalanine with phenolic acids, cleavage of the amine protecting group of compounds **12a** and **12b** was carried out. Thus, the 4-hydroxybenzylamide of *N*-(*tert*-butyloxycarbonyl) dehydrophenyalanine was treated with TFA. Removal of the solvent at reduced pressure gave the 4-hydroxybenzylamide of dehydrophenyalanine trifluorate (compound **14a**, Scheme 23) in 99% yield.



Scheme 23 – Synthesis of the 4-hydroxybenzylamide and the dopamide of dehydrophenylalanine trifluorate.

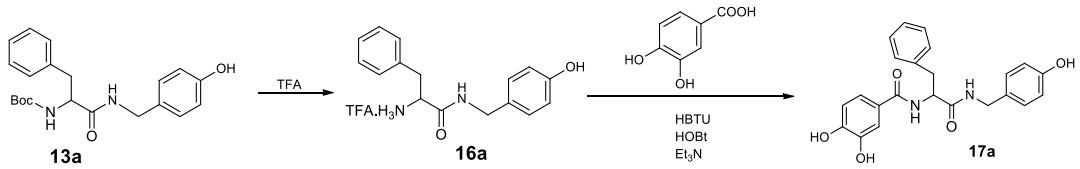
The same reaction using compound **12b** as substrate gave the corresponding dopamide derivative in quantitative yield (compound **14b**, Scheme 23).

The unprotected dehydrophenylalanine derivatives could now be reacted with a phenolic acid to give phenolic acid-dehydrophenylalanine-amine conjugates. Thus, compound **14a** was reacted with protocatechuic acid using HOBt/HBTU as coupling agents to give the 4-hydroxybenzylamide of *N*-protocatechoyl dehydrophenylalanine (compound **15a**, Scheme 24) as a yellow oil in 75% yield.



Scheme 24 – Synthesis of the 4-hydroxybenzylamide of *N*-protocatechoyl dehydrophenylalanine.

The corresponding phenylalanine derivative of compound **15a** was obtained by treatment of compound **13a** with TFA to give compound **16a** (Scheme 25) as a white solid in 85% yield. Coupling of **16a** with protocatechuic acid gave the 4-hydroxybenzylamide of *N*-protocatechoyl phenylalanine (compound **17a**, Scheme 25) as a light yellow solid in 65% yield.



Scheme 25 – Synthesis of the 4-hydroxybenzylamide of *N*-protocatechoyl phenylalanine.

Thus, a wide range of hydroxyphenylamides of *N*-phenoyl dehydrophenylalanine and phenylalanine can be obtained in good yields by coupling *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine with phenolic or catecholic amines. Subsequently, these dehydrophenylalanine derivatives are submitted to cleavage of the Boc group and coupling with phenolic acids (Table 5).

Table 5 – Yields obtained in the synthesis of hydroxyphenylamides of *N*-acyl dehydrophenylalanine and phenylalanine derivatives.

Reagent	Product	Yield (%)
Boc-ΔPhe-OH, **12**	Boc-ΔPhe-NH-Bzl(4-OH), **12a**	88
Boc-Phe-OH, **13**	Boc-Phe-NH-Bzl(4-OH), **13a**	94
Boc-ΔPhe-OH, **12**	Boc-ΔPhe-NH-Dopa, **12b**	97
Boc-Phe-OH, **13**	Boc-Phe-NH-Dopa, **13b**	91
Tfa.H-ΔPhe-NH-Bzl(4-OH), **14a**	Bz(3,4-OH)-ΔPhe-NH-Bzl(4-OH), **15a**	75
Tfa.H-Phe-NH-Bzl(4-OH), **16a**	Bz(3,4-OH)-Phe-NH-Bzl(4-OH), **17a**	65

2.3.4. Synthesis of the 4-hydroxybezylamide of a *N*-acyl dehydrotripeptide

Some drugs are ubiquitous in the management of many diseases and injuries. However, even these well-established medications can cause stomach ulcers and other gastrointestinal disorders. Side effects most commonly arise when the drugs are taken for an extended period. One way of preventing these painful consequences is to encapsulate drugs to restrict their availability in certain parts of the body and target their release to others.

Small peptides with aromatic side chains, aromatic *N*-acyl groups or aromatic amides have been known to self-assemble into gel type structures such as hydrogels. These hydrogels have been studied as vehicles for drug loading and release, such as anti-inflammatory agents. One such agent is naproxen. It is well-established that naproxen covalently bound to peptides plays an adjuvant role in self-assembly driven by hydrophobic interactions in water. Naproxen is so successful in co-assembly due to its structure with two fused aromatic rings (Figure 14), as well as similar derivatives of the naphthalene unit.[97]

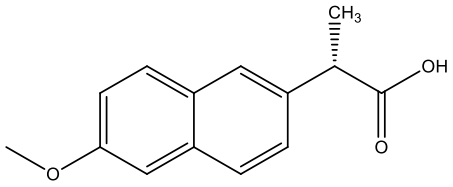
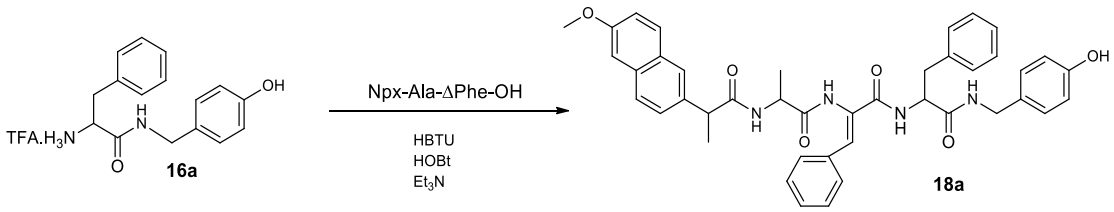


Figure 14 – Structure of Naproxen.

In order to obtain a tripeptide baring a dehydrophenylalanine residue, a phenolic amide and naproxen as acylating group, compound **16a** was reacted with *N*-naproxoyl-alanil-dehydrophenylalanine. The tripeptide Npx-Ala-ΔPhe-Phe-NH-Bzl(4-OH) (compound **18a**, Scheme 26) was obtained as a white solid in 70% yield.



Scheme 26 – Synthesis of Npx-Ala-ΔPhe-Phe-NH-Bzl(4-OH).

3. Conclusions and future perspectives

In this work, the synthesis of phenolic and catecholic Ugi adducts and the corresponding amides of *N*-acyl (hydroxyphenyl)glycines is described.

The Ugi adducts were obtained in high yields (83%-97%) by the Ugi reaction using different *O*-(*tert*-butyloxycarbonyl) benzaldehydes, namely, *tert*-butyl (4-formylphenyl) carbonate, *tert*-butyl (4-formyl-2-methoxyphenyl) carbonate and di-*tert*-butyl (4-formyl-1,2-phenylene) dicarbonate; and different carboxylic acids, namely, benzoic acid, acetic acid, 2-naphtylacetic acid, 4-hydroxyphenylacetic acid, *p*-coumaric acid, protocatechuic acid, 3,4-dihydroxyphenylacetic acid, hydrocaffeic acid and *N*-benzyloxycarbonylglycine.

An attempt to carry out the condensation reaction using di-*tert*-butyl (3-formyl-1,2-phenylene) dicarbonate and benzoic acid gave only 17% of the corresponding Ugi adduct. This was attributed to the position of the bulky *tert*-butyloxycarbonyl group which causes high steric hindrance.

The Ugi adducts were treated with TFA, which led to removal of the hydroxyl protecting *tert*-butyloxycarbonyl group and of the 4-methoxybenzyl group. However, the cyclohexylamide was maintained. Thus, the corresponding cyclohexylamides of (hydroxyphenyl)glycine were obtained in yields between 34% and 80%. The stability of the cyclohexylamide relatively to treatment with neat TFA seems to result from the steric effect produced by the phenyl group directly linked to the α–carbon. This steric effect is comparable to that reported for the α,α-dibenzyl moiety in *N*-acyl-*N*,α,α-trialkylglycine amides.[95]

The DPPH method was used to study and evaluate the antiradical capacity of some of the compounds synthesized. The results show a high increase of activity with the increase in number of hydroxyl or catechol groups. The monocatecholic derivatives have twice the radical-scavenging activity of protocatechuic acid, while the dicatecholic derivatives have almost four times this activity. Thus, the introduction of the catecholic moiety in an amino acid structure induces a significant increase in its intrinsic radical scavenging activity.

The synthesis of hydroxyphenylamides of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine was also carried out. To obtain an *N*-acyl dehydrophenyalanine derivative that could be coupled with phenolic or catecholic amines, *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine was prepared. Then, *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine was coupled with a phenolic or a catecholic amine to give the 4-hydroxybenzylamide or the dopamide of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine, respectively. The corresponding derivatives of the saturated amino acid phenylalanine were also prepared. These compounds were obtained in yields between 88% and 97%.

To enable the synthesis of hydroxyphenylamides of *N*-protocatechoyl dehydrophenylalanine, cleavage of the amine protecting group with TFA was carried out to give the 4-hydroxybenzylamide of dehydrophenylalanine trifluorate. Then, the dehydrophenylalanine derivative was coupled with protocatechuic to give the 4-hydroxybenzylamide of *N*-(protocatechoyl) dehydrophenylalanine. The corresponding derivative of the saturated amino acid phenylalanine was also prepared.

Thus, it was possible to prepare phenoyl and catechoyl amides of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine and subsequently, in the case of the 4-hydroxybenzylamide dehydrophenylalanine derivative, conjugate it with protocatechuic acid.

These novel compounds can have important biological activities resulting from the synergic effects of both the catechol and the dehydro moieties. However, due to time constraints, it was not possible to perform biological tests for any of these compounds. Thus, as future perspectives, it is proposed to test the antioxidant/antiradicalar activity of the compounds obtained, to understand if the dehydro moiety has a high influence on the antioxidant activity.

A 4-hydroxybenzylamide tripeptide baring a dehydrophenylalanine residue and having naproxen as *N*-acyl group was also synthesized. This was carried out by coupling *N*-naproxoyl-alanil-dehydrophenylalanine with the 4-hydroxybenzylamide of phenylalanine to give the 4-hydroxybenzylamide of a *N*-naproxoyl dehydrotripeptide in 70% yield.

It was not possible to perform any tests with this compound, but it is known that naproxen is an excellent anti-inflammatory agent. It can be inserted into peptide-based hydrogels with potential in various bioengineering applications such as vehicles for transporting and releasing drugs into the body. As future perspectives tests should be carried out to confirm if this tripeptide can be used for this purpose.

4. Experimental Section

4.1.General methods

Melting points (°C) were determined in a Gallenkamp apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II+ at 400 and 100.6 MHz, respectively. ^{1}H-^{1}H spin-spin decoupling, DEPT θ 45°, HMQC and HMBC were used to attribute some signals. In the description of each NMR spectrum, the data are indicated in the following order: chemical shift (δ) in parts per million (ppm), multiplicity of signal [s (singlet), d (doublet), q (quartet), m multiplet)], coupling constant (*J*) in Hertz (Hz), number of protons (nH), proton identification. The chemical shifts indicated for each compound were recorded relative to the internal standard which is comprised of the solvent used for spectrum acquisition. HRMS data were recorded by the Laboratory for Structural Elucidation of the Materials Centre of the University of Porto on an LTQ Orbitrap™ XL hybrid mass spectrometer (Thermo Fischer Scientific, Bremen, Germany) controlled by *LTQ Tune Plus 2.5.5* and *Xcalibur 2.1.0*. Elemental analysis was performed on a LECO CHNS 932 elemental analyzer. The reactions were monitored by thin layer chromatography (TLC). Column chromatography was performed on Macherey-Nagel silica gel 230-400 mesh. Petroleum ether refers to the boiling range 40-60 °C. Most of the solvents used had a purity of p.a., so they were used without prior purification. The exception was acetonitrile which was dried by stirring with silica at room temperature overnight, refluxed with calcium hydride and distilled into a vial with drying agents.

4.2. DPPH Radical Essay

DPPH radical-scavenging activity was assessed according to standard procedures.[60,96] A methanolic DPPH· stock solution (1.93 mmol.dm^{-3}) was diluted to give a 0.10 mmol dm^{-3} working solution. The reaction between DPPH· and each AO was monitored at 515 nm by using a Powerwave XS Microplate Reader (Bio-Tek Instruments, Inc) thermostated at T = 25.0 ± 0.1°C. The wells of a 96-well microplate contained a methanolic solution of the AO (3-200 μmol.dm^{-3}) and 80 μmol.dm^{-3} DPPH·. The absorbance of each well was recorded at 1 min intervals for a 60 min period. The absorbance of each solution was subtracted from the blank (80 μmol.dm^{-3} DPPH· without AO). The antiradical activity was defined as the relative concentration of antioxidant required to lower the initial DPPH concentration by 50% [EC50 (mol/L phenolic compound per unit DPPH concentration)].

4.3. Statistical analysis

All the DPPH• radical scavenging assays were run at least in quadruplicate. SPSS 21.0 software was used for statistical analysis by one-way analysis of variance (ANOVA, with Tukey's HSD multiple comparison) with the level of significance set at $P<0.05$. Data are presented as means ± standard deviation.

4.4. Synthesis

4.4.1. Synthesis of phenolic and catecholic amino acid derivatives by the modified multicomponent Ugi reaction

Procedure A: Synthesis of *O-tert*-butyloxycarbonylated benzaldehydes.

To a solution of the hydroxybenzaldehyde (5.00 mmol) in dry acetonitrile (0.20 $mol.dm^{-3}$), 0.10 equiv. of dimethylaminopyridine was added. This was followed by addition under rapid stirring at room temperature of 1.10 equiv. of *tert*-butyldicarbonate for the monohydroxylated benzaldehydes and 2.20 equiv. for the dihydroxylated benzaldehydes. When all the reactant had been fully *tert*-butyloxycarbonylated the solvent was evaporated at reduced pressure and the residue dissolved in ethyl acetate (100 cm^3) and washed with $KHSO_4$ (1 $mol.dm^{-3}$) and brine (3 times 25 cm^{-3} each). The organic layer was dried with $MgSO_4$ and the solvent evaporated at reduced pressure.

Procedure B: Two step synthesis of the cyclohexylamides of *N*-acyl, (hydroxyphenyl)glycine.

To a solution of the *O-tert*-butyloxycarbonylated benzaldehyde (1.00 mmol) in ethanol/2,2,2-trifluoroethanol (1/1) (0.17 $mol.dm^{-3}$) under a stream of nitrogen, 1.10 equiv. of 4-methoxybenzylamine was added. After stirring for 4 hours, 1.10 equiv. of the carboxylic acid and 1.10 equiv. of cyclohexyl isocyanide were added and left to react for 2 days. The reaction mixture was then evaporated at reduced pressure and the residue was dissolved in ethyl acetate (100 cm^3) and washed with $KHSO_4$ (1 $mol.dm^{-3}$), $NaHCO_3$ (1 $mol.dm^{-3}$) and brine (3 times 25 cm^3 each). The organic layer was dried with $MgSO_4$ and the solvent evaporated at reduced pressure to give the corresponding Ugi adduct. Trifluoroacetic acid was added to the adduct (0.25 $mol.dm^{-3}$) and the solution refluxed at 80 °C for 10 min. TFA was then evaporated at reduced pressure.

Procedure C: One-pot synthesis of of the cyclohexylamides of *N*-acyl (hydroxyphenyl)glycine cyclohexylamides.

To a solution of the *O-tert*-butyloxycarbonylated benzaldehyde (1.00 mmol) in ethanol/2,2,2-trifluoroethanol (1/1) (0.17 mol.dm^{-3}), 1.10 equiv. of 4-methoxybenzylamine was added under a stream of nitrogen. After 4 hours, 1.10 equiv. of the carboxylic acid and 1.10 equiv. of cyclohexyl isocyanide were added and left to react for 2 days. The reaction mixture was then evaporated at reduced pressure and trifluoroacetic acid added to the residue (0.25 mol.dm^{-3}). The solution was refluxed at 80 °C for 10 min. after which TFA was evaporated at reduced pressure.

4.1.1.1. Synthesis of *O-tert*-butyloxycarbonylated benzaldehydes

Synthesis of *tert*-butyl (4-formylphenyl) carbonate, a[92]

Procedure A was followed using 4-hydroxybenzaldehyde to give compound **a** (1.07 g, 96%) as a white solid (from diethyl ether/*n*-hexane). M.p. 73.0-74.0 °C.

^{1}H NMR (400 MHz, CDCl3): δ = 1.58 [s, 9H, C(CH$_3$)$_3$], 6.36 (d, *J* = 6.8 Hz, 2H, ArH), 7.92 (d, *J* = 6.8 Hz, 2H, ArH), 10.00 (s, 1H, CHO) ppm.

Synthesis of *tert*-butyl (4-formyl-2-methoxyphenyl) carbonate, b[93]

Procedure A was followed using 4-hydroxy-3-methoxybenzaldehyde to give compound **b** (1.20 g, 95%) as a white solid (from diethyl ether/*n*-hexane). M.p. 87.0-88.0 °C.

^{1}H NMR (400 MHz, CDCl3): δ = 1.57 [s, 9H, C(CH$_3$)$_3$], 3.94 (s, 3H, OCH$_3$), 7.31 (d, *J* = 8.0 Hz, 1H, ArH), 7.48 (dd, *J* = 8.0 Hz, *J* = 2.0 Hz, 2H, ArH), 7.51 (d, *J* = 2.0 Hz, 1H, ArH), 9.95 (s, 1H, CHO) ppm

Synthesis of di-*tert*-butyl (4-formyl-1,2-phenylene) dicarbonate, c[94]

Procedure A was followed using 3,4-dihydroxybenzaldehyde to give compound **c** (1.57 g, 93%) as a light brown oil.

^{1}H NMR (400 MHz, CDCl$_3$): δ = 1.55 [s, 18H, 2C(CH$_3$)$_3$], 7.45 (d, *J* = 8.0 Hz, 1H, ArH), 7.77 (dd, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H, ArH), 7.80 (d, *J* = 1.6 Hz, 1H, ArH), 9.95 (s, 1H, CHO) ppm.

Synthesis of di-*tert*-butyl (3-formyl-1,2-phenylene) dicarbonate, d

Procedure A was followed using 2,3-dihydroxybenzaldehyde was followed to give compound **d** (1.62 g, 96%) as a light brown solid. M.p. 58.0-59.0 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.56 [s, 18H, 2C(CH_3)$_3$], 7.36-7.40 (m, 1H, ArH), 7.53 (dd, J = 8.0 Hz, J = 1.6 Hz, 1H, ArH), 7.75 (dd, J = 8.0 Hz, J = 1.6 Hz, 1H, ArH), 10.19 (s, 1H, CHO) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 27.11 [C(CH3)3], 27.14 [C(CH3)3], 83.94 [C(CH3)3], 84.37 [C(CH3)3], 126.07 (CH), 126.39 (CH), 128.53 (CH), 129.35 (C), 143.08 (C), 143.74 (C) 149.67 (C=O), 149.99 (C=O), 187.27 (CH=O) ppm.

HRMS (ESI): m/z M+ calcd for $C_{17}H_{22}O_7$: 338.1366; found: 338.1597.

4.4.1.2. Synthesis of Ugi adducts

Synthesis of the cyclohexylamide of *N*-benzoyl, *N*-(4-metoxybenzylamino)-[4-*O*-*tert*-butyloxycarbonyl)phenyl]glycine, 1a'

Procedure B was followed using *tert*-butyl (4-formylphenyl) carbonate and benzoic acid to give the Ugi adduct **1a'** (0.54 g, 94%) as a white solid (from diethyl ether). M.p. 65.0-66.0 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.86-1.93 (m, 10H, CH_2 cyclohexyl), 1.57 [s, 9H, C(CH_3)$_3$], 3.76 (s, 3H, OCH_3), 3.79-3.82 (m, 1H, CH cyclohexyl), 4.41 (br. s, 1H, $C_6H_4CH_2$), 4.63 (br. s, 1H, $C_6H_4CH_2$), 5.40 (br. s, 1H, αCH), 5.74 (br. s, 1H, NH), 6.74 (d, *J* = 8.4 Hz, 2H, ArH), 7.00 (br. d, *J* = 4.8 Hz, 2H, ArH), 7.13 (d, *J* = 8.8 Hz, 2H, ArH), 7.35-7.37 (m, 4H, ArH), 7.46-7.49 (m, 3H, ArH) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 24.70 (CH_2), 24.78 (CH_2), 25.44 (CH_2), 27.66 [C(*C*H_3)$_3$], 32.69 (2CH_2), 48.64 (CH cyclohexyl), 55.20 (OCH_3), 64.08 (αCH), 65.82 ($C_6H_4CH_2$), 83.80 [*C*(CH_3)$_3$], 113.87 (3CH), 121.61 (3CH), 126.68 (CH), 128.38 (CH), 128.49 (CH), 129.84 (CH), 130.07 (CH), 130.70 (CH), 132.65 (2C), 133.35 (CH), 136.08 (C), 151.08 (C), 151.54 (C), 158.76 (C=O), 168.04 (C=O), 173.07 (C=O) ppm.

Anal. calcd. for $C_{34}H_{40}N_2O_6$ (572.69): C 71.31, H 7.04, N 4.89; found C 71.39, H 7.32, H 4.51.

Synthesis of the cyclohexylamide of *N*-benzoyl, *N*-(4-metoxybenzylamino)-[2,3-di(*O*-*tert*-butyloxycarbonyl)phenyl]glycine, 1d'

Procedure B was followed using di-*tert*-butyl (3-formyl-1,2-phenylene) dicarbonate and benzoic acid. The residue obtained was chromatographed through silica using ethyl acetate/petroleum ether as eluent to give the Ugi adduct **1d'** (0.12 g, 17%) as a light yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.15-1.96 (m, 10H, CH_2 cyclohexyl), 1.46, 1.57 [2s, 18H, C(CH_3)$_3$], 3.79 (br. s, 4H, OCH_3 + CH cyclohexyl), 4.25 (d, *J* = 4.8 Hz, 2H, $C_6H_4CH_2$), 4.80 (br. s, 1H, αCH),

5.92 (br. d, *J* = 7.6 Hz, 1H, NH), 6.86 (d, *J* = 8.8 Hz, 2H, ArH), 6.94 (t, *J* = 8.0 Hz, 1H, ArH), 7.14 (dd, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H, ArH), 7.18-7.22 (m, 3H, ArH), 7.47 (t, *J* = 8.0 Hz, 2H, ArH), 7.59 (t, *J* = 7.2 Hz, 1H, ArH), 8.01 (dd, *J* = 8.0 Hz, *J* = 1.2 Hz, 2H, ArH) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 24.59 (CH_2), 24.69 (CH_2), 25.47 (CH_2), 27.57 [C(*C*H_3)$_3$], 28.38 [C(*C*H_3)$_3$], 32.91 (2CH_2), 48.06 (CH cyclohexyl), 55.26 (OCH_3), 58.43 (C_6H_4*C*H_2), 82.02 [2*C*(CH_3)$_3$], 113.96 (2CH), 119.46 (CH), 120.20 (CH), 120.56 (C), 121.56 (CH), 124.29 (CH), 128.49 (2CH), 128.79 (CH), 129.49 (2CH), 129.62 (C), 130.51 (C), 130.87 (CH), 130.94 (C), 130.97 (C), 133.18 (CH), 144.88 (C), 158.89 (2C=O), 168.82 (C=O), 172.00 (C=O) ppm.

HRMS (ESI): m/z M^+ calcd for $C_{39}H_{48}N_2O_9$: 688.3360; found: 688.3968.

Synthesis of the cyclohexylamide of *N*-acetyl, *N*-(4-metoxybenzylamino)-[4-(*O*-*tert*-butyloxycarbonyl)phenyl]glycine, 2a'

Procedure B was followed using *tert*-butyl (4-formylphenyl) carbonate and acetic acid to give the Ugi adduct **2a'** in quantitative yield as a light yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.01-1.15 (m, 3H, CH_2 cyclohexyl), 1.20-1.48 (m, 3H, CH_2 cyclohexyl), 1.54 [s, 9H, C(CH_3)$_3$], 1.56-1.69 (m, 2H, CH_2 cyclohexyl), 1.81-1.93 (m, 2H, CH_2 cyclohexyl), 2.08 (s, 3H, CH_3CO), 3.68-3.78 (m, 4H, NHCH + OCH_3), 4.46 (d, *J* = 17.2 Hz, 1H, NCH_2), 4.65 (d, *J* = 17.2 Hz, 1H, NCH_2), 5.83 (br. s, 2H, αCH + NH), 6.74 (d, *J* = 8.4 Hz, 2H, ArH), 6.91 (d, *J* = 8.4 Hz, 2H, ArH), 7.08 (d, *J* = 8.4 Hz, 2H, ArH), 7.36 (d, *J* = 8.4 Hz, 2H, ArH) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 22.36 (CH_3), 24.65 (CH_2), 24.71 (CH_2), 25.36 (CH_2), 27.58 [C(*C*H_3)$_3$], 32.61 (2CH_2), 48.60 (CH cyclohexyl), 50.35 ($C_6H_4CH_2$), 55.13 (OCH_3), 62.04 (αCH), 83.73 [*C*(CH_3)$_3$], 113.83 (2CH), 121.44 (2CH), 127.32 (2CH), 129.08 (C), 130.70 (2CH), 132.68 (C), 150.98 (C), 151.47 (C), 158.56 (C=O), 168.43 (C=O), 172.63 (C=O) ppm.

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{29}H_{39}N_2O_6$: 511.2808; found: 511.2796.

Synthesis of the cyclohexylamide of *N*-(4-hydroxyphenyl)acetyl, *N*-(4-methoxybenzylamino)-[4-(*O*-*tert*-butyloxycarbonyl), 3-methoxyphenyl]glycine, 4b'

Procedure B was followed using *tert*-butyl (4-formyl-2-methoxyphenyl) carbonate and 4-hydroxyphenylacetic acid to give the Ugi adduct **4b'** (0.61 g, 97%) as a white solid (from ethyl acetate/petroleum ether). M.p. 141.0-143.0 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.05-1.10 (m, 3H, CH_2 cyclohexyl), 1.25-1.32 (m, 3H, CH_2 cyclohexyl), 1.54 [s, 9H, C(CH_3)$_3$], 1.57-1.63 (m, 2H, CH_2 cyclohexyl), 1.87-1.89 (m, 2H, CH_2

cyclohexyl), 3.57-3.69 (m, 3H, NHC*H* + CH_2 acetyl), 3.76 (s, 3H, OCH_3), 4.48 (br. d, *J* = 17.2 Hz, 1H, $C_6H_4CH_2$), 4.71 (br. d, *J* = 17.2 Hz, 1H, $C_6H_4CH_2$), 5.75 (s, 1H, NH), 5.92 (br. s, 1H, αCH), 6.66 (d, *J* = 8.4 Hz, 2H, ArH), 6.75 (d, *J* = 8.4 Hz, 2H, ArH), 6.90-7.01 (m, 7H, ArH) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 24.18 (CH_2), 24.66 (CH_2), 24.74 (CH_2), 25.38 (CH_2), 27.58 [C(*C*H_3)$_3$], 32.55 (CH_2), 40.35 (*C*$H_2C_6H_3$), 48.85 (CH cyclohexyl), 55.22 (OCH_3), 55.92 (OCH_3), 60.40 ($C_6H_4CH_2$), 83.66 [*C*(CH_3)$_3$], 113.96 (2CH), 114.02 (CH), 115.68 (2CH), 122.03 (CH), 122.55 (2CH), 125.64 (C), 127.51 (2CH), 129.05 (C), 130.00 (2CH), 133.64 (C), 140.25 (C), 151.23 (C), 155.48 (C), 158.72 (C), 168.56 (C=O), 171.21 (C=O), 173.78 (C=O) ppm.

Synthesis of the cyclohexylamide of *N*-(4-hydroxyphenyl)acetyl, *N*-(4-metoxybenzylamino)-[3,4-(*O*-di-*tert*-butyloxycarbonyl)phenyl]glycine, 4c'

Procedure B was followed using di-*tert*-butyl (4-formyl-1,2-phenylene) dicarbonate and 4-hydroxyphenylacetic acid to give the Ugi adduct **4c'** (0.66 g, 92%) as a light brown oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.05-1.11 (m, 3H, CH_2 cyclohexyl), 1.25-1.33 (m, 3H, CH_2 cyclohexyl), 1.54 [s, 18H, 2C(CH_3)$_3$], 1.57-1.63 (m, 2H, CH_2 cyclohexyl), 1.86-1.89 (m, 2H, CH_2 cyclohexyl), 3.57-3.67 (m, 2H, CH_2 acetyl), 3.75-3.80 (m, 4H, NHCH + OCH_3), 4.48 (br. d, *J* = 17.2 Hz, 1H, $C_6H_4CH_2$), 4.72 (br. d, *J* = 17.2 Hz, 1H, $C_6H_4CH_2$), 5.80 (s, 1H, αCH), 5.97 (br. d, *J* = 5.6 1H, NH), 6.67 (d, *J* = 8.0 Hz, 2H, ArH), 6.74 (d, *J* = 8.0 Hz, 2H, ArH), 6.90 (d, *J* = 8.0 Hz, 2H, ArH), 6.97 (d, *J* = 7.6 Hz, 2H, ArH), 7.10-7.14 (m, 2H, ArH), 7.32 (s, 1H, ArH) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 24.66 (CH_2), 24.74 (2CH_2), 25.36 (CH_2), 27.58 [C(*C*H_3)$_3$], 32.47 (CH_2), 40.30 (*C*$H_2C_6H_4$), 48.97 (CH cyclohexyl), 55.19 (OCH_3), 60.41 ($C_6H_4CH_2$), 83.98 [*C*(CH_3)$_3$], 84.01 [*C*(CH_3)$_3$], 114.03 (2CH), 114.09 (CH), 115.71 (2CH), 123.18 (CH), 124.87 (CH), 125.59 (C), 127.50 (CH), 127.61 (CH), 127.77 (CH), 128.73 (C), 130.03 (2CH), 133.46 (C), 142.47 (C), 150.45 (C), 155.37 (C), 158.74 (C), 168.23 (2C=O), 173.79 (2C=O) ppm.

Synthesis of the cyclohexylamide of *N*-*p*-coumaroyl, *N*-(4-methoxybenzylamino)-[4-(*O*-*tert*-butyloxycarbonyl), 3-methoxyphenyl]glycine, 5b'

Procedure B was followed using *tert*-butyl (4-formyl-2-methoxyphenyl) carbonate and *p*-coumaric acid to give the Ugi adduct **5b'** (0.53 g, 83%) as a white solid (from ethyl acetate/petroleum ether). M.p. 131.0-133.0 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.08-1.13 (m, 3H, CH_2 cyclohexyl), 1.24-1.33 (m, 3H, CH_2 cyclohexyl), 1.54 [s, 9H, C(CH_3)$_3$], 1.56-1.64 (m, 2H, CH_2 cyclohexyl), 1.76-1.90 (m, 2H, CH_2 cyclohexyl), 3.64-3.81 (m, 7H, NHC*H* + 2OCH_3), 4.43 (br. d, *J* = 17.2 Hz, 1H, $C_6H_4CH_2$), 4.67 (br.

d, *J* = 17.2 Hz, 1H, $C_6H_4CH_2$), 5.76 (s, 1H, NH), 5.99 (br. s, 1H, αCH), 6.42 (d, *J* = 15.2 Hz, 1H, Ar-CH=CH), 6.67 (d, *J* = 8.4 Hz, 2H, ArH), 6.73-6.91 (m, 5H, ArH), 7.00 (d, *J* = 7.6 Hz, 2H, ArH), 7.14 (d, *J* = 8.0 Hz, 2H, ArH), 7.59 (d, *J* = 15.2 Hz, 1H, Ar-CH=CH) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 24.15 (CH_2), 24.63 (CH_2), 24.71 (CH_2), 25.36 (CH_2), 27.57 [$C(CH_3)_3$], 32.53 (CH_2), 49.00 (CH cyclohexyl), 55.18 (OCH_3), 55.84 (OCH_3), 60.41 ($C_6H_4CH_2$), 83.59 [$C(CH_3)_3$], 113.85 (2CH), 114.15 (CH), 114.39 (CH), 116.07 (CH), 116.17 (2CH), 122.07 (CH), 122.50 (CH), 126.63 (C), 127.60 (2CH), 129.85 (2CH), 133.37 (C), 140.27 (C), 144.21 (CH), 155.11 (C), 158.60 (C), 158.80 (C), 158.90 (C), 168.71 (C=O), 169.41 (C=O), 171.22 (C=O) ppm.

Synthesis of the cyclohexylamide of *N*-(protocatechoyl), *N*-(4-metoxybenzylamino)-[4-(*O*-*tert*-butyloxycarbonyl)]glycine, 6a'

Procedure B was followed using *tert*-butyl (4-formylphenyl) carbonate and protocatechuic acid to give the Ugi adduct **6a'** in quantitative yield as a colourless oil that solidified on standing. M.p. 119.0-120.0 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.03-1.86 (m, 10H, CH_2 cyclohexyl), 1.57 [s, 9H, $C(CH_3)_3$], 3.72-3.75 (m, 4H, OCH_3 + CH cyclohexyl), 4.39 (br. s, 1H, $C_6H_4CH_2$), 4.76 (br. s, 1H, $C_6H_4CH_2$), 5.16 (br. s, 1H, αCH), 5.81 (br. s, 1H, NH), 6.72-6.79 (m, 3H, ArH), 6.89 (br. d, *J* = 8.4 Hz, 1H, ArH), 7.00-7.02 (m, 2H, ArH), 7.10-7.12 (m, 3H, ArH), 7.32 (br. d, *J* = 8.4 Hz, 2H, ArH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 24.60 (CH_2), 24.66 (CH_2), 25.32 (CH_2), 27.67 [$C(CH_3)_3$], 32.46 ($2CH_2$), 48.94 (CH cyclohexyl), 55.21 (OCH_3), 60.40 (αCH), 83.89 [$C(CH_3)_3$], 113.96 (3CH), 114.86 (CH), 115.05 (CH), 119.67 (CH), 121.70 (3CH), 126.75 (C), 128.68 (CH), 130.03 (C), 130.60 (CH), 132.22 (C), 144.34 (C), 147.07 (C), 151.18 (C), 151.55 (C), 158.87 (C=O), 168.84 (C=O), 171.18 (C=O) ppm.

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{34}H_{41}N_2O_8$: 605.2863; found: 605.2856.

Synthesis of the cyclohexylamide of *N*-(protocatechoyl), *N*-(4-metoxybenzylamino)-[4-(*O*-*tert*-butyloxycarbonyl), 3-methoxyphenyl]glycine, 6b'

Procedure B was followed using *tert*-butyl (4-formyl-2-methoxyphenyl) carbonate and protocatechuic acid to give the Ugi adduct **6b'** in quantitative yield as a white solid. M.p. 158.0-159.0 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.04-1.86 (m, 10H, CH_2 cyclohexyl), 1.56 [s, 9H, $C(CH_3)_3$], 3.72-3.74 (m, 7H, $2OCH_3$ + CH cyclohexyl), 4.42 (br. s, 1H, $C_6H_4CH_2$), 4.73 (br. s, 1H, $C_6H_4CH_2$), 5.10

(br. s, 1H, αCH), 5.90 (br. s, 1H, NH), 6.73 (d, *J* = 8.0 Hz, 2H, ArH), 6.77 (br. d, *J* = 8.0 Hz, 2H, ArH), 6.87-6.88 (m, 3H, ArH), 6.99 (br. d, *J* = 8.0 Hz, 2H, ArH), 7.04 (d, *J* = 8.0 Hz, 2H, ArH), 7.10 (br. s, 1H, ArH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 24.59 (CH_2), 24.66 (CH_2), 25.33 (CH_2), 27.60 [C(*C*H_3)$_3$], 32.46 (2CH_2), 48.89 (CH cyclohexyl), 55.20 (OCH_3), 55.90 (OCH_3), 60.39 ($C_6H_4CH_2$), 83.70 [*C*(CH_3)$_3$], 113.91 (3CH), 114.75 (CH), 115.06 (CH), 119.55 (CH), 121.87 (3CH), 122.70 (CH), 126.93 (C), 128.69 (CH), 133.58 (C), 140.33 (C), 144.41 (C), 146.99 (C), 151.28 (C), 151.36 (C), 158.84 (C=O), 168.75 (C=O), 173.77 (C=O) ppm.

HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{35}H_{43}N_2O_9$: 635.29686; found: 635.29800.

Synthesis of the cyclohexylamide of *N*-(3,4-dihydroxyphenyl)acetyl, *N*-(4-methoxybenzylamino)-[4-(*O*-*tert*-butyloxycarbonyl), 3-methoxyphenyl]glycine, 7b'

Procedure B was followed using *tert*-butyl (4-formyl-2-methoxyphenyl) carbonate and 3,4-dihydroxyphenylacetic acid to give the Ugi addcut **7b'** (0.61g, 93%) as a white solid (from ethyl acetate/petroleum ether). M.p. 149.0-152.0 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.06-1.82 (m, 10H, CH_2 cyclohexyl), 1.54 [s, 9H, C(CH_3)$_3$], 3.57-3.66 (m, 5H, OCH_3 + CH_2CO), 3.69-3.78 (m, 4H, NHC*H* + OCH_3), 4.44 (br. d, *J* = 16.0 Hz, 1H, $C_6H_4CH_2$), 4.71 (br. d, *J* = 16.0 Hz, 1H, $C_6H_4CH_2$), 5.48 (br. s, 1H, NH), 5.67 (br. s, 1H, αCH), 6.51 (br. d, *J* = 7.2 Hz, 1H, H), 6.71-6.79 (m, 5H, ArH), 6.83-7.01 (m, 5H, ArH) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 24.24 (CH_2), 24.68 (CH_2), 24.74 (CH_2), 25.33 (CH_2), 27.57 [C(*C*H_3)$_3$], 32.50 (CH_2), 40.63 (*C*$H_2C_6H_3$), 48.92 (CH cyclohexyl), 55.22 (OCH_3), 55.88 (OCH_3), 60.39 ($C_6H_4CH_2$), 83.75 [*C*(CH_3)$_3$], 114.00 (2CH), 114.16 (CH), 115.26 (CH), 115.75 (CH), 120.96 (CH), 121.99 (CH), 122.52 (CH), 125.94 (C), 127.60 (CH), 127.83 (CH), 128.72 (C), 133.45 (C), 140.24 (C), 143.70 (C), 144.37 (C), 151.23 (C), 151.34 (C), 158.80 (C=O), 168.65 (C=O), 174.12 (C=O) ppm.

Synthesis of the cyclohexylamide of *N*-(3,4-dihydroxyphenyl)acetyl, *N*-(4-metoxybenzylamino)-[3,4-(*O*-di-*tert*-butyloxycarbonyl)phenyl]glycine, 7c'

Procedure B was followed using di-*tert*-butyl (4-formyl-1,2-phenylene) dicarbonate and 3,4-dihydroxyphenylacetic acid to give the Ugi adduct **7c'** (0.63g, 86%) as a light yellow solid. M.p. 109.0-110.0 °C

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.08-1.86 (m, 10H, CH_2 cyclohexyl), 1.56 [s, 18H, 2C(CH_3)$_3$], 3.63-3.82 (m, 6H, OCH_3 + CH_2CO + NHC*H*), 4.36 (br. d, *J* = 16.4 Hz, 1H, $C_6H_4CH_2$), 4.68 (br. d, *J* =

16.4 Hz, 1H, $C_6H_4CH_2$), 5.53 (br. s, 1H, αCH), 5.95 (br. s, 1H, NH), 6.50 (br. d, *J* = 7.2 Hz, 1H, ArH), 6.68-6.88 (m, 5H, ArH), 6.94-7.48 (m, 5H, ArH + NH) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 24.24 (CH_2), 24.65 (CH_2), 25.36 (CH_2), 27.62 [C(CH_3)$_3$], 29.69 (CH_2), 32.49 (CH_2), 40.88 ($CH_2C_6H_3$), 49.04 (CH cyclohexyl), 55.24 (OCH_3), 63.81 ($C_6H_4CH_2$), 84.19 [C(CH_3)$_3$], 84.41 [C(CH_3)$_3$], 114.14 (2CH), 114.29 (CH), 115.20 (CH), 115.86 (CH), 121.04 (CH), 123.21 (CH), 123.29 (CH), 123.54 (C), 124.84 (CH), 126.02 (C), 127.98 (CH), 128.21 (CH), 142.56 (C), 143.14 (C), 143.61 (C), 144.13 (C), 150.55 (C), 150.79 (C), 158.97 (C=O), 167.94 (C=O), 173.15 (C=O), 173.56 (C=O) ppm.

Synthesis of the cyclohexylamide of *N*-benzyloxycarbonylglycyl, *N*-(4-metoxybenzylamino)-[3,4-(*O*-di-*tert*-butyloxycarbonyl)phenyl]glycine, 9c'

Procedure B was followed using 3,4-di(*O*-*tert*-butyloxycarbonyl)benzaldehyde and *N*-benzyloxycarbonylglycine to give compound **9c'** (1.12g, 94%) as a light pink solid (from ethyl acetate/petroleum ether). M.p. 113.0-114.0 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.10-1.12 (m, 3H, CH_2 cyclohexyl), 1.31-1.34 (m, 3H, CH_2 cyclohexyl), 1.56 [s, 18H, 2C(CH_3)$_3$], 1.65-1.70 (m, 2H, CH_2 cyclohexyl), 1.84-1.88 (m, 2H, CH_2 cyclohexyl), 3.74-3.80 (m, 4H, OCH_3 + CH cyclohexyl), 3.93 (br. d, *J* = 16.8 Hz, 1H, αCH_2), 4.10 (br. d, *J* = 16.8 Hz, 1H, αCH_2), 4.43 (br. d, *J* = 17.2 Hz, 1H, ArCH_2), 4.60 (br. d, *J* = 17.2 Hz, 1H, ArCH_2), 5.10 (s, 2H, CH_2 Z), 5.66 (d, *J* = 7.6 Hz, 1H, αCH), 5.71-5.73 (m, 2H, 2NH), 6.75 (d, *J* = 8.4 Hz, 2H, ArH), 6.94 (d, *J* = 8.4 Hz, 2H, ArH), 7.15 (s, 2H, ArH), 7.30-7.35 (m, 6H, ArH) ppm.

^{13}C NMR (100 6 MHz, $CDCl_3$): δ = 22.66 (CH_2), 24.73 (CH_2), 24.81 (CH_2), 25.40 (CH_2), 27.58 [2C(CH_3)$_3$], 29.63 (CH_2), 32.65 (CH_2), 43.46 (αCH_2), 48.91 (CH cyclohexyl), 49.27 (ArCH_2), 55.17 (OCH_3), 62.75 (αCH), 66.85 (CH_2Z), 84.03 [2C(CH_3)$_3$], 114.17 (CH), 123.37 (CH), 125.00 (CH), 127.69 (CH), 127.95 (2CH), 128.03 (2CH), 128.46 (3CH), 133.04 (C), 136.39 (C), 142.59 (C), 142.75 (C), 150.39 (C), 150.43 (C), 156.14 (C=O), 158.91 (2C=O), 167.37 (C=O), 170.12 (C=O) ppm.

HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{42}H_{54}N_3O_{11}$: 776.37583; found: 776.37679.

4.4.1.3. Synthesis of the cyclohexylamides of *N*-acyl (hydroxyphenyl)glycine

Synthesis of the cyclohexylamide of *N*-benzoyl, (4-hydroxyphenyl)glycine, 1a

Procedure B was followed using Ugi adduct **1a'** to give compound **1a** (0.12 g, 34%) as a white solid (from diethyl ether). Alternatively, procedure C using *tert*-butyl (4-formylphenyl) carbonate and benzoic acid was followed to give comound **1a** (0.22g, 62%). M.p. 141.0-142.0 °C.

^{1}H NMR (400 MHz, CD_3OCD_3): δ = 1.14-1.36 (m, 5H, CH_2 cyclohexyl), 1.57-2.12 (m, 5H, CH_2 cyclohexyl), 3.68-3.76 (m, 1H, CH cyclohexyl), 5.64 (s, 1H, αCH), 6.82 (d, *J* = 8.4 Hz, 2H, ArH), 7.37 (d, *J* = 8.4 Hz, 2H, ArH), 7.46-7.50 (m, 2H, ArH), 7.53-7.57 (m, 1H, ArH), 7.93-7.95 (m, 2H, ArH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 25.48 (CH_2), 25.57 (CH_2), 26.22 (CH_2), 33.21 (CH_2), 33.30 (CH_2), 49.10 (CH cyclohexyl), 57.27 (αCH), 115.91 (2CH), 128.08 (2CH), 129.17 (2CH), 129.58 (2H), 131.04 (C), 132.13 (CH), 135.40 (C), 157.83 (C), 166.36 (C=O), 170.12 (C=O) ppm.

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{21}H_{25}N_2O_3$: 353.1865; found: 353.1856.

Synthesis of the cyclohexylamide of *N*-benzoyl, (4-hydroxy-3-methoxyphenyl)glycine, 1b

Procedure C was followed using *tert*-butyl (4-formyl-2-methoxyphenyl) carbonate and benzoic acid to give compound **1b** (0.29g, 76%) as a light yellow solid (from diethyl ether). M.p. 169.0-170.0 °C.

^{1}H NMR (400 MHz, CD_3OCD_3): δ = 1.15-1.36 (m, 5H, CH_2 cyclohexyl), 1.57-1.91 (m, 5H, CH_2 cyclohexyl), 3.69-3.75 (m, 1H, CH cyclohexyl), 3.83 (s, 3H, OCH_3), 5.67 (s, 1H, αCH), 6.81 (d, *J* = 8.0 Hz, 1H, ArH), 7.01 (dd, *J* = 8.0 Hz, *J* = 2.0 Hz, 1H, ArH), 7.19 (d, *J* = 2.0 Hz, 1H, ArH), 7.46-7.57 (m, 3H, ArH), 7.94-7.96 (m, 2H, ArH), 8.04 (s, 1H, NH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 25.48 (CH_2), 25.56 (CH_2), 26.22 (CH_2), 33.21 (CH_2), 33.29 (CH_2), 49.09 (CH cyclohexyl), 56.23 (CH_3), 57.60 (αCH), 111.94 (CH), 115.53 (CH), 121.15 (CH), 128.12 (2CH), 129.16 (2CH), 131.49 (C), 132.13 (CH), 135.43 (C), 147.05 (C), 148.17 (C), 166.46 (C=O), 170.07 (C=O) ppm.

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{22}H_{27}N_2O_4$: 383.1971; found: 383.1973.

Synthesis of the cyclohexylamide of *N*-benzoyl, (3,4-dihydroxyphenyl)glycine, 1c

Procedure C was followed using di-*tert*-butyl (4-formyl-1,2-phenylene) dicarbonate and benzoic acid. The residue obtained after removal of TFA was chromatographed through silica using ethyl acetate/petroleum ether as eluent to give compound **1c** (0.24g, 66%) as a white solid. M.p. 127.0-128.0 °C.

^{1}H NMR (400 MHz, CD_3OCD_3): δ = 1.28-1.36 (m, 5H, CH_2 cyclohexyl), 1.57-1.92 (m, 5H, CH_2 cyclohexyl), 3.69-3.77 (m, 1H, CH cyclohexyl), 5.59 (d, *J* = 7.6 Hz, 1H, αCH), 6.80 (d, *J* = 8.4

Hz, 1H, ArH), 6.88 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H, ArH), 7.04 (d, *J* = 2.0 Hz, 1H, ArH), 7.33 (d, *J* = 7.6 Hz, 1H, C_6H_5CONH), 7.46-7.55 (m, 3H, ArH), 7.90-7.97 (m, 5H, 2ArH + 2OH + NHC_6H_{11}) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 25.49 (CH_2), 25.57 (CH_2), 26.22 (CH_2), 33.24 (CH_2), 33.32 (CH_2), 49.23 (CH cyclohexyl), 57.53 (αCH), 115.58 (CH), 115.91 (CH), 119.97 (CH), 128.07 (2CH), 129.17 (2CH), 131.73 (C), 132.12 (CH), 135.42 (C), 145.66 (C), 145.77 (C), 166.39 (C=O), 170.17 (C=O) ppm.

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{21}H_{25}N_2O_4$: 369.1814; found: 369.1811.

Synthesis of the cyclohexylamide of *N*-acetyl, (4-hydroxyphenyl)glycine, 2a

Procedure B was followed using Ugi adduct **2a'** to give compound **2a** as a white solid (from acetone). M.p. 241.0-242.0 °C.

^{1}H NMR (400 MHz, DMSO): δ = 1.16-1.24 (m, 5H, CH_2 cyclohexyl), 1.49-1.75 (m, 5H, CH_2 cyclohexyl), 1.85 (s, 3H, CH_3CO), 3.40-3.45 (m, 1H, NHCH), 5.31 (d, *J* = 8.4 Hz, 1H, αCH), 6.66 (d, *J* = 8.4 Hz, 2H, ArH), 7.15 (d, *J* = 8.4 Hz, 2H, ArH), 7.99 (d, *J* = 8.0 Hz, 1H, NH), 8.29 (d, *J* = 8.4 Hz, 1H, NH), 9.34 (s, 1H, OH) ppm.

^{13}C NMR (100.6 MHz, DMSO): δ = 22.46 (CH_3), 24.37 (CH_2), 24.49 (CH_2), 25.17 (CH_2), 32.18 (CH_2), 32.30 (CH_2), 47.52 (CH cyclohexyl), 55.38 (αCH), 114.88 (2CH), 128.11 (2CH), 129.62 (C), 156.59 (C), 168.69 (C=O), 169.29 (C=O) ppm.

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{16}H_{23}N_2O_3$: 291.1709; found: 291.1705.

Synthesis of the cyclohexylamide of *N*-(2-Naphthyl)acetyl, (3,4-dihydroxyphenyl)glycine, 3c

Procedure C was followed using di-*tert*-butyl (4-formyl-1,2-phenylene) dicarbonate and 2-naphthylacetic acid. The residue obtained after removal of TFA was dissolved in ethyl acetate (100 cm^3) and washed with $KHSO_4$ (1 $mol.dm^{-3}$), $NaHCO_3$ (1 $mol.dm^{-3}$) and brine (3 times 25 cm^3 each). The organic layer was dried with $MgSO_4$ and the solvent evaporated at reduced pressure to give compound **3c** (0.34g, 78%) as a light yellow solid (from ethyl acetate/dichloromethane). M.p. 220.0-221.0 °C.

^{1}H NMR (400 MHz, CD_3OCD_3): δ = 1.06-1.33 (m, 5H, CH_2 cyclohexyl), 1.63-1.83 (m, 5H, CH_2 cyclohexyl), 3.59-3.66 (m, 1H, CH cyclohexyl), 3.81 [d, *J* = 2.8 Hz, CH_2 (2-naphthyl)acetyl], 5.38 (d, *J* = 8.0 Hz, 1H, αCH), 6.75 (s, 2H, ArH), 6.95 (s, 1H, ArH), 7.17 (d, *J* = 8.0 Hz, 1H, C_6H_3CONH), 7.46-7.53 (m, 3H, ArH), 7.70 (d, *J* = 7.6 Hz, 1H, NHC_6H_{11}), 7.84-7.89 (m, 6H, ArH + 2OH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 25.42 (CH_2), 25.52 (CH_2), 26.18 (CH_2), 33.19 (CH_2), 33.28 (CH_2), 43.57 [CH_2 (2-naphthyl)acetyl], 49.01 (CH cyclohexyl), 57.28 (αCH), 115.46 (CH), 115.84 (CH), 119.79 (CH), 126.32 (CH), 126.80 (CH), 128.40 (2CH), 128.54 (CH), 128.59 (CH), 128.65 (CH), 131.80 (C), 133.29 (C), 134.49 (C), 134.85 (C), 145.57 (C), 145.74 (C), 170.04 (C=O), 170.25 (C=O) ppm.

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{26}H_{29}N_2O_4$: 433.2127; found: 433.2131.

Synthesis of the cyclohexylamide of *N*-(4-hydroxyphenyl)acetyl, (4-hydroxy, 3-methoxyphenyl)glycine, 4b

Procedure B was followed using Ugi adduct **4b'** to give compound **4b** (0.53g, 59%) as a light pale solid (from ethyl acetate/petroleum ether). M.p. 165.0-167.0 °C.

^{1}H NMR (400 MHz, CD_3OCD_3): δ = 1.23-1.31 (m, 5H, CH_2 cyclohexyl), 1.56-1.72 (m, 4H, CH_2 cyclohexyl), 1.81-1.85 (m, 1H, CH_2 cyclohexyl), 3.52 (t, *J* = 2.8 Hz, 2H, CH_2 acetyl), 3.56-3.66 (m, 1H, CH cyclohexyl), 3.76 (s, 3H, OCH_3), 5.43 (s, 1H, αCH), 6.75 (d, *J* = 8.0 Hz, 2H, ArH), 6.79 (d, *J* = 8.0 Hz, 2H, ArH), 6.84 (dd, *J* = 2.0 Hz, *J* = 0.4 Hz, 1H, ArH), 6.87 (dd, *J* = 2.0 Hz, *J* = 0.4 Hz, 1H, ArH), 6.99 (d, *J* = 2.0 Hz, 1H, ArH), 7.19 (d, *J* = 8.4 Hz, 1H ArH) ppm. ^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 25.43 (CH_2), 25.53 (CH_2), 26.20 (CH_2), 33.17 (CH_2), 33.25 (CH_2), 42.70 (CH_2 acetyl), 48.92 (CH cyclohexyl), 56.13 (OCH_3), 57.04 (αCH), 111.46 (CH), 115.44 (CH), 115.96 (2CH), 120.62 (CH), 127.70 (C), 131.08 (2CH), 131.62 (C), 146.85 (C), 148.06 (C), 157.02 (C), 169.97 (C=O), 170.85 (C=O) ppm.

Synthesis of the cyclohexylamide of *N*-(4-hydroxyphenyl)acetyl, (3,4-dihydroxyphenyl)glycine, 4c

Procedure B was followed using Ugi adduct **4c'** (0.359 g, 0.500 mmol). The residue obtained was chromatographed through silica using ethyl acetate/petroleum ether as eluent to give compound **4c** (0,16 g, 80.2%) as a light orange oil.

^{1}H NMR (400 MHz, CD_3OCD_3): δ = 1.24-1.32 (m, 5H, CH_2 cyclohexyl), 1.54-1.72 (m, 4H, CH_2 cyclohexyl), 1.81-1.83 (m, 1H, CH_2 cyclohexyl), 3.52 (s, 2H, CH_2 acetyl), 3.60-3.78 (m, 1H, CH cyclohexyl), 5.39 (d, *J* = 7.6 Hz, 1H, αCH), 6.75-6.80 (m, 4H, ArH), 6.94 (s, 1H, ArH), 7.16 (d, *J* = 8.4 Hz, 2H, ArH), 7.32 (br. d, *J* = 8.0 Hz, 1H, NH), 7.63 (d, *J* = 7.6 Hz, 1H, NH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 25.41 (CH_2), 25.51 (CH_2), 26.16 (CH_2), 33.14 (CH_2), 33.21 (CH_2), 42.62 (CH_2 acetyl), 49.11 (CH cyclohexyl), 57.23 (αCH), 115.34 (CH), 115.83 (CH), 116.06 (2CH), 119.98 (CH), 127.47 (C), 131.10 (2CH), 131.53 (C), 145.58 (C), 145.74 (C), 157.08 (C), 170.28 (C=O), 171.30 (C=O) ppm.

Synthesis of the cyclohexylamide of *N*-protocatechoyl, (4-hydroxyphenyl)glycine, 6a

Procedure B was followed using Ugi adduct **6a'** (0,454 g, 0.750 mmol). The residue obtained after removal of TFA was dissolved in ethyl acetate (100 cm^3) and washed with $KHSO_4$ (1 $mol.dm^{-3}$), $NaHCO_3$ (1 $mol.dm^{-3}$) and brine (3 times 25 cm^3 each). The organic layer was dried with $MgSO_4$ and the solvent evaporated at reduced pressure to give compound **6a** (0.21 g, 72%) as a white solid (from ethyl acetate/dichloromethane). M.p. 158.0-159.0 °C.

^{1}H NMR (400 MHz, CD_3OCD_3): δ = 1.11-1.39 (m, 5H, CH_2 cyclohexyl), 1.57-1.78 (m, 4H, CH_2 cyclohexyl), 1.87-1.90 (m, 1H, CH_2 cyclohexyl), 3.69-3.75 (m, 1H, CH cyclohexyl), 5.60 (d, J = 7.6 Hz, 1H, αCH), 6.80-6.82 (m, 2H, ArH), 6.89 (d, J = 8.0 Hz, 1H, ArH), 7.34-7.36 (m, 3H, ArH), 7.46 (d, J = 2.0 Hz, 1H, ArH), 7.75 (d, J = 7.6 Hz, 1H, NH), 8.42 (br. s, 3H, OH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 25.48 (CH_2), 25.56 (CH_2), 26.21 (CH_2), 33.22 (CH_2), 33.29 (CH_2), 49.21 (CH cyclohexyl), 57.29 (αCH), 115.50 (CH), 115.61 (CH), 115.92 (2CH), 120.38 (CH), 127.16 (C), 129.53 (2CH), 131.30 (C), 145.60 (C), 149.20 (C), 157.79 (C), 166.23 (C=O), 170.41 (C=O) ppm.

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{21}H_{25}N_2O_5$: 385.17635; found: 385.16979.

Synthesis of the cyclohexylamide of *N*-protocatechoyl, (4-hydroxy-3-methoxyphenyl)glycine, 6b

Procedure B was followed using Ugi adduct **6b'** (0,636 g, 1.000 mmol). The residue obtained after removal of TFA was dissolved in ethyl acetate (100 cm^3) and washed with $KHSO_4$ (1 $mol.dm^{-3}$), $NaHCO_3$ (1 $mol.dm^{-3}$) and brine (3 times 25 cm^3 each). The organic layer was dried with $MgSO_4$ and the solvent evaporated at reduced pressure to give to give compound **6b** (0.28 g, 67.5%) as a white solid (from ethyl acetate/dichloromethane). M.p. 129.0-130.0 °C.

^{1}H NMR (400 MHz, CD_3OCD_3): δ = 1.17-1.35 (m, 5H, CH_2 cyclohexyl), 1.58-1.89 (m, 5H, CH_2 cyclohexyl), 3.68-3.73 (m, 1H, CH cyclohexyl), 3.81 (s, 3H, OCH_3), 5.63 (d, J = 7.2 Hz, 1H, αCH), 6.80 (d, J = 8.0 Hz, 1H, ArH), 6.89 (d, J = 8.4 Hz, 1H, ArH), 6.99 (dd, J = 8.4 Hz, J = 2.0 Hz, 1H, ArH), 7.17 (d, J = 2.0 Hz, 1H, ArH), 7.36 (dd, J = 8.4, J = 2.0 Hz, 1H, ArH), 7.40 (br. s., 1H, NH), 7.48 (d, J = 2.0 Hz, 1H, ArH), 7.81 (d, J = 7.2 Hz, 1H, NH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 25.48 (CH_2), 25.56 (CH_2), 26.21 (CH_2), 33.24 (CH_2), 33.30 (CH_2), 49.20 (CH cyclohexyl), 56.22 (CH_3), 57.66 (αCH), 111.91 (CH), 115.58 (2CH), 115.62 (CH), 120.44 (CH), 121.07 (CH), 127.15 (C), 131.70 (C), 145.63 (C), 147.05 (C), 148.16 (C), 149.25 (C), 166.41 (C=O), 170.39 (C=O) ppm.

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{22}H_{27}N_2O_6$: 415.18691; found: 415.18636.

Synthesis of the cyclohexylamide of *N*-protocatechoyl, (3,4-dihydroxyphenyl)glycine, 6c

Procedure C was followed using di-*tert*-butyl (4-formyl-1,2-phenylene) dicarbonate and protocatechuic acid. The residue obtained after removal of TFA was chromatographed through silica using ethyl acetate/petroleum ether as eluent to give compound **6c** (0.29 g, 69%) as a white solid (from ethyl acetate/dichloromethane). M.p. 177.0-178.0 °C.

^{1}H NMR (400 MHz, CD_3OCD_3): δ = 1.21-1.33 (m, 6H, CH_2 cyclohexyl), 1.57-1.91 (m, 4H, CH_2 cyclohexyl), 3.71-3.74 (m, 1H, CH cyclohexyl), 5.55 (d, *J* = 7.6 Hz, 1H, αCH), 6.78 (d, *J* = 8.0 Hz, 1H, ArH), 6.85 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H, ArH), 6.90 (d, *J* = 8.0 Hz, 1H, ArH), 7.03 (d, *J* = 2.0 Hz, 1H, ArH), 7.35 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H, ArH), 7.48 (d, *J* = 2.0 Hz, 1H, ArH), 7.72 (d, *J* = 7.2 Hz, 1H, C_6H_3CONH), 7.95 (br. s, 1H, OH), 8.08 (br. s, 1H, OH), 8.45 (br. s, 1H, OH), 8.49 (br. s, 1H, OH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 25.49 (CH_2), 25.57 (CH_2), 26.23 (CH_2), 33.21 (CH_2), 33.27 (CH_2), 49.23 (CH cyclohexyl), 57.35 (αCH), 115.54 (CH), 115.86 (CH), 119.84 (2CH), 120.36 (2CH), 127.07 (C), 127.10 (C), 131.91 (C), 131.94 (C), 145.65 (C), 149.26 (C), 166.17 (C=O), 170.34 (C=O) ppm.

HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{21}H_{24}N_2NaO_6$: 423.1532; found: 423.1528.

Synthesis of the cyclohexylamide of *N*-(3,4-dihydroxyphenyl)acetyl, (4-hydroxy, 3-methoxyphenyl)glycine, 7b

Procedure B was followed using Ugi adduct **7b'** (0.324 g, 0.500 mmol). The residue obtained was chromatographed through silica using ethyl acetate/petroleum ether as eluent to give compound **7b** (0.095 g, 44%) as a white solid (from ethyl acetate/petroleum ether). M.p. 150.0-152.0 °C.

^{1}H NMR (400 MHz, CD_3OCD_3): δ = 1.21-1.35 (m, 5H, CH_2 cyclohexyl), 1.57-1.85 (m, 5H, CH_2 cyclohexyl), 3.45 (s, 2H, CH_2 acetyl), 3.63-3.65 (m, 1H, CH cyclohexyl), 3.71 (s, 3H, OCH_3), 5.34 (br. s, 1H, αCH), 6.69 (dd, *J* = 8.0 Hz, *J* = 2.0 Hz, 1H, ArH), 6.74-6.88 (m, 3H, ArH), 6.96 (s, 1H, ArH), 7.04 (d, *J* = 8.8 Hz, 1H, ArH), 7.23 (br. s, 2H, OH), 7.47 (br. s, 1H, OH), 7.56 (d, *J* = 9.6 Hz, 1H, ArH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 25.43 (CH_2), 25.53 (CH_2), 26.19 (CH_2), 33.18 (CH_2), 33.27 (CH_2), 40.67 ($CH_2C_6H_3$), 49.02 (CH cyclohexyl), 56.22 (CH_3), 56.16 (αCH), 111.42 (CH), 114.48 (CH), 115.54 (CH), 116.01 (CH), 117.15 (CH), 118.12 (CH), 118.57 (CH), 120.65 (CH), 121.42

(CH), 126.13 (C), 128.44 (C), 130.61 (CH), 131.65 (C), 132.54 (C), 133.81 (C), 144.21 (C), 144.88 (C), 145.90 (C), 146.99 (C), 148.16 (C), 158.87 (C), 170.04 (C=O), 170.83 (C=O) ppm.

Synthesis of the cyclohexylamide of *N*-(3,4-dihydroxyphenyl)acetyl, (3,4-dihydroxyphenyl)glycine, 7c

Procedure B was followed using Ugi adduct **7c'** (0,367 g, 0.500 mmol). The residue obtained was chromatographed through silica using ethyl acetate/petroleum ether as eluent to give compound **7c** (0.131 g, 63.4%) as a brown solid. M.p. 115.0-116.0 °C.

^{1}H NMR (400 MHz, CD_3OCD_3): δ = 1.24-1.34 (m, 5H, CH_2 cyclohexyl), 1.59-1.72 (m, 4H, CH_2 cyclohexyl), 1.81-1.88 (m, 1H, CH_2 cyclohexyl), 3.46 (s, 2H, CH_2 acetyl), 3.63-3.80 (m, 1H, CH cyclohexyl), 5.33 (d, *J* = 7.6 Hz, 1H, αCH), 6.67 (dd, *J* = 8.0 Hz, *J* = 2.0 Hz, 1H, ArH), 6.71-6.83 (m, 4H, ArH), 6.87 (d, *J* = 2.0 Hz, 1H, ArH), 6.91 (d, *J* = 1.2 Hz, 1H, ArH), 7.22 (br. d, *J* = 7.6 Hz, 1H, NH), 7.50 (d, *J* = 7.6 Hz, 1H, NH), 8.06 (s, 1H, OH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 25.43 (CH_2), 25.53 (CH_2), 26.19 (CH_2), 33.17 (CH_2), 33.25 (CH_2), 42.95 (CH_2 acetyl), 49.08 (CH cyclohexyl), 57.24 (αCH), 115.36 (CH), 115.90 (CH), 117.17 (CH), 119.69 (CH), 121.47 (CH), 128.26 (C), 130.63 (CH), 131.60 (C), 144.81 (C), 145.54 (C), 145.69 (C), 145.80 (C), 170.16 (C=O), 171.08 (C=O) ppm.

Synthesis of the cyclohexylamide of *N*-hydrocaffeoyl, (3,4-dihydroxyphenyl)glycine, 8c

Procedure C was followed using di-*tert*-butyl (4-formyl-1,2-phenylene) dicarbonate and hydrocaffeic acid. The residue obtained after removal of TFA was chromatographed through silica using ethyl acetate/petroleum ether as eluent to give **8c** (0.32 g, 74%) as a white solid. M.p. 112.0-113.0 °C.

^{1}H NMR (400 MHz, CD_3OD): δ = 1.18-1.36 (m, 6H, CH_2 cyclohexyl), 1.61-1.90 (m, 4H, CH_2 cyclohexyl), 2.32-2.54 (m, 2H, CH_2), 2.72-2.77 (m, 2H, CH_2), 3.72-3.88 (m, 1H, CH cyclohexyl), 5.23 (s, 1H, αCH), 6.54 (s, 1H, ArH), 6.64-6.68 (m, 2H, ArH), 6.74-6.68 (m, 5H, ArH), 7.02 (d, *J* = 8.4 Hz, 2H, ArH) ppm.

^{13}C NMR (100.6 MHz, CD_3OD): δ = 26.06 (CH_2), 26.12 (CH_2), 26.59 (CH_2), 29.27 (CH_2), 33.47 (CH_2), 33.5 (CH_2), 38.28 (CH_2), 49.97 (CH cyclohexyl), 58.28 (αCH), 114.77 (CH), 115.70 (CH), 116.37 (CH), 117.49 (CH), 118.69 (CH), 120.23 (CH), 130.31 (C), 130.59 (CH), 131.47 (C), 131.68 (C), 134.96 (C), 144.61 (C), 146.46 (C), 159.31 (C), 171.97 (C=O), 174.80 (C=O) ppm. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{23}H_{29}N_2O_6$: 429.2026; found: 429.2030.

Synthesis of the cyclohexylamide of *N*-benzyloxycarbonylglycyl, (3,4-dihydroxyphenyl)glycine, 9c

Procedure B was followed using Ugi adduct **9c'** (0.58 g, 0.75 mmol) to give **9c** (0.25 g, 74%) as a white solid (from ethyl acetate/diethyl ether). M.p. 162.0-163.0 °C.

^{1}H NMR (400 MHz, CD_3OCD_3): δ = 1.11-1.71 (m, 10H, CH_2 cyclohexyl), 3.63-3.67 (m, 1H, CH cyclohexyl), 3.87 (d, *J* = 6.0 Hz, 2H, αCH_2), 5.10 (s, 2H, CH_2 Z), 5.32 (d, *J* = 7.6 Hz, 1H, αCH), 6.70 (br. d, *J* = 7.6 Hz, 1H, NH), 6.73 (s, 2H, ArH), 6.91 (s, 1H, ArH), 7.26-7.39 (m, 6H, ArH + NH), 7.66 (d, *J* = 7.2 Hz, 1H, NH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 25.62 (CH_2), 26.27 (CH_2), 29.26 (CH_2), 33.26 (CH_2), 33.38 (CH_2), 45.01 (αCH_2), 49.20 (CH cyclohexyl), 57.16 (αCH), 66.93 (CH_2Z), 115.45 (CH), 115.90 (CH), 119.80 (CH), 128.63 (2CH), 129.24 (3CH), 131.60 (C), 138.16 (C), 145.66 (C), 145.78 (C), 157.63 (C=O), 169.13 (C=O), 170.00 (C=O) ppm.

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{24}H_{30}N_3O_6$: 456.2135; found: 456.2141.

4.5. Synthesis of phenolic and catecholic derivatives of dehydrophenylalanine

Procedure D: Synthesis of the phenoyl amides of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine and phenylalanine.

To a solution of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine or phenylalanine in acetonitrile (0.01 mol.dm^{-3}), 1.0 equiv. of HOBt was added, followed by 1.0 equiv. of HBTU, 1.0 equiv. of amine and 1.1 equiv. of NEt_3 in an ice bath. After stirring for 4 hours at room temperature, the solvent was evaporated at reduced pressure. The residue was dissolved in ethyl acetate (100 cm^3) and washed with $KHSO_4$ (1 mol.dm^{-3}), $NaHCO_3$ (1.mol dm^{-3}) and brine (3 times 25 cm^3 each). The organic layer was dried with $MgSO_4$ and the solvent evaporated at reduced pressure to give the corresponding compound.

Procedure E: Cleavage of the amine protecting group.

To a hydroxyphenylamide of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine and phenylalanine, 6 cm^3 of trifluroacetic acid (TFA) were added and the solution left to stand for 30 min. The solvent was evaporated at reduced pressure and the residue triturated with diethyl ether and filtered to give the corresponding hydroxyphenylamide of dehydrophenylalanine and phenylalanine.

Procedure F: Coupling of unprotected dehydrophenylalanine or phenylalanine derivatives with a phenolic acid.

To a solution of the hydroxyphenylamide of dehydrophenylalanine or phenylalanine in acetonitrile (0.0100 mol.dm^{-3}), 1.0 equiv. of HOBt was added, followed by 1.0 equiv. of HBTU, 1.0 equiv. of protocatechuic acid and 2.2 equiv. of NEt_3 in an ice bath. After stirring overnight at room temperature, the solvent was evaporated at reduced pressure. The residue was dissolved in ethyl acetate (40 cm^3) and washed with $KHSO_4$ (1 mol.dm^{-3}), $NaHCO_3$ (1 mol.dm^{-3}) and brine (3 times 10 cm^3 each). The organic layer was dried with $MgSO_4$ and the solvent evaporated at reduced pressure to give the corresponding hydroxyphenylamide of *N*-phenoyl dehydrophenylalanine or phenylalanine.

4.5.1. Synthesis of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine

Synthesis of methyl ester of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine, Boc-ΔPhe-OMe, 11

To a solution of **Boc-Phe(*β*-OH)-OMe** (1.48 g, 5.000 mmol) in dry acetonitrile (0.5 mol.dm^{-3}), 0.11 equiv. of DMAP was added followed by 1.1 equiv. of *tert*-butylpyrocarbonate under rapid stirring at room temperature. The reaction was monitored by TLC (diethyl ether/ *n*-hexane, 1:1) until all the reactant had been consumed. Then 2% in volume of TMG was added, stirring was continued and the reaction followed by TLC. When all the reactant was consumed evaporation at reduced pressure gave a residue that was partitioned between 100 cm^3 of ethyl acetate and 25 cm^3 of $KHSO_4$ (1 mol.dm^{-3}). The organic phase was washed with $KHSO_4$ (1 mol.dm^{-3}), $NaHCO_3$ (1 mol.dm^{-3}) and brine (2 times 30 cm^3 each) and dried over $MgSO_4$. Removal of the solvent afforded compound **11** (1.36 g, 98%) as a white solid.[90]

^{1}HNMR (400 MHz, $CDCl_3$): 1.40 (s, 9H, CH_3 Boc), 3.86 (s, 3H, OCH_3), 6.17 (s, 1H, βH), 7.26 (br. s, 1H, NH), 7.30-7.39 (m, 3H, ArH), 7.54 (d, *J* = 7.2 Hz, 2H, ArH) ppm.

Synthesis of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine, Boc-ΔPhe-OH, 12

To a solution of compound **11** (0.69 g, 2.500 mmol.) in dioxane (0.2 mol.dm^{-3}), 7.5 cm^3 of NaOH (1 mol.dm^{-3}) was added under rapid stirring at room temperature. The reaction was monitored by TLC. When the reaction was complete, $KHSO_4$ was added until pH 3. The aqueous solution was extracted with ethyl acetate (5 times 15 cm^3), the organic fractions were collected and washed with brine (1 time 25 cm^3) and dried over $MgSO_4$. Removal of the solvent gave compound **12** (0.63 g, 95%) as a white solid. (from ethyl acetate/petroleum ether). M.p. 161.0-162.0 °C.

^{1}HNMR (400 MHz, DMSO): δ = 1.36 (s, 9H, CH_3 Boc), 7.16 (s, 1H, βH), 7.31-7.41 (m, 3H, ArH), 7.62 (d, *J* = 7.2 Hz, 2H, ArH), 8.46 (br. s, 1H, NH), 12.64 (br. s, 1H, OH) ppm.
^{13}CNMR (100.6 MHz, DMSO): δ = 28.03 [C(*C*H_3)$_3$], 78.89 [*C*(CH_3)$_3$], 127.13 (CH), 128.44 (2CH), 129.03 (CH), 129.77 (2CH), 131.37 (C), 133.91 (C), 153.76 (C=O), 166.88 (C=O) ppm.
HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{14}H_{18}NO_4$: 264.12358; found: 264.12323.

4.5.2. Synthesis of the hydroxyphenylamides of *N*-(*tert*-butyloxycarbonyl) dehydrophenylalanine and phenylalanine

Synthesis of the 4-hydroxybenzylamide of *N*-(*tert*-butyloxycarbonyl) dehydrophenyalanine, Boc-ΔPhe-NH-Bzl(4-OH), 12a

Procedure D was followed using compound **12** (0.62 g, 2.350 mmol) and 4-hydroxybenzylamine to give compound **12a** (0.76 g, 88%) as a yellow oil.
^{1}HNMR (400 MHz, DMSO): δ = 1.38 (br. s, 9H, CH_3 Boc), 4.24 (d, *J* = 6.0 Hz, 2H, CH_2 Bzl), 6.68 (d, *J* = 8.4 Hz, 2H, ArH Bzl), 6.97 (br. s, 1H, NH), 7.11 (d, *J* = 8.4 Hz, 2H, ArH Bzl), 7.27-7.38 (m, 4H, ArH Phe + βCH), 7.53 (d, *J* = 7.6 Hz, 2H, ArH Phe), 8.39 (br. s, 1H, NH), 9.22 (s, 1H, OH) ppm.
^{13}C NMR (100.6 MHz, DMSO): δ = 28.03 [C(*C*H_3)$_3$], 42.15 (CH_2 Bzl), 78.86 [*C*(CH_3)$_3$], 114.82 (2CH), 128.30 (2CH), 128.36 (2CH), 128.60 (CH), 128.71 (CH), 129.29 (2CH), 129.80 (C), 134.48 (C), 153.50 (C), 156.11 (C), 165.34 (C=O), 170.33 (C=O) ppm. m/z (HRESI-MS).
HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{21}H_{25}N_2O_4$: 369.18143; found: 369.18199.

Synthesis of the dopamide of *N*-(*tert*-butyloxycarbonyl)dehydrophenyalanine, Boc-ΔPhe-NH-Dopa, 12b

Procedure D was followed using compound **12** (0.62 g, 2.350 mmol) and dopamine hydrochloride to give compound **12b** (0.91 g, 97%) as a light yellow solid.
^{1}HNMR (400 MHz, CD_3OCD_3): δ = 1.41 (s, 9H, CH_3 Boc), 2.75 (t, *J* = 7.6 Hz, 2H, CH_2), 3.49-3.54 (m, 2H, CH_2), 6.61 (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H, ArH Dopa), 6.77 (d, *J* = 8.0 Hz, 1H, ArH Dopa), 6.78 (d, *J* = 2.0 Hz, 1H, ArH Dopa), 7.13 (s, 1H, βCH), 7.31-7.49 (m, 5H, ArH Phe + 2NH), 7.58 (d, *J* = 7.6 Hz, 2H, ArH), 7.72 (br. s, 1H, OH), 7.79 (br. s, 1H, OH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 28.39 [C(*C*H$_3$)$_3$], 35.88 (CH_2 Dopa), 42.30 (CH_2 Dopa), 80.23 [*C*(CH$_3$)$_3$], 116.05 (CH), 116.58 (CH), 120.82 (CH), 129.12 (CH), 129.24 (2CH), 129.34 (C), 130.21 (3CH), 132.04 (C), 135.78 (C), 144.33 (C), 145.87 (C), 154.34 (C=O), 166.05 (C=O) ppm.

HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{22}H_{27}N_2O_5$: 399.19200; found: 399.19216.

Synthesis of the 4-hydroxybenzylamide of *N*-(*tert*-butyloxycarbonyl) phenyalanine, Boc-Phe-NH-Bzl(4-OH), 13a

Procedure D was followed using compound *N*-(*tert*-butyloxycarbonyl) phenylalanine (0.66 g, 2.500 mmol) and 4-hydroxybenzylamine to give compound **13a** (0.87 g, 94%) as a white solid (from ethyl acetate/petroleum ether). M.p. 123.0-124.0 °C.

^{1}HNMR (400 MHz, DMSO): δ = 1.29 (s, 9H, CH$_3$ Boc), 2.68-2.77 (m, 1H, βCH$_2$), 2.91-2.95 (m, 1H, βCH$_2$), 4.14-4.19 (m, 3H, CH$_2$Bzl + αCH), 6.44 (br. d, *J* = 7.6 Hz, 1H, NH), 6.66 (d, *J* = 8.4 Hz, 2H, ArH Bzl), 6.89 (d, *J* = 8.4 Hz, 1H, ArH Phe), 6.98 (d, *J* = 8.0 Hz, 2H, ArH Bzl), 7.17-7.19 (m, 1H, ArH Phe), 7.23-7.26 (m, 3H, ArH Phe), 8.24 (t, *J* = 5.6 Hz, 1H, NH), 9.26 (s, 1H, OH) ppm.

^{13}C NMR (100.6 MHz, DMSO): δ = 28.15 [C(*C*H$_3$)$_3$], 37.63 (βCH$_2$), 41.65 (CH$_2$ Bzl), 55.89 (αCH), 78.02 [*C*(CH$_3$)$_3$], 114.94 (2CH), 126.17 (CH), 128.03 (2CH), 128.43 (2CH), 129.22 (2CH), 139.35 (C), 138.15 (C), 155.23 (C), 156.19 (C=O), 171.47 (C=O) ppm.

HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{21}H_{27}N_2O_4$: 371.19708; found: 371.19794.

Synthesis of the dopamide of *N*-(*tert*-butyloxycarbonyl) phenyalanine, Boc-Phe-NH-Dopa, 13b

Procedure D was followed using compound *N*-(*tert*-butyloxycarbonyl) phenylalanine (0.66 g, 2.500 mmol) and dopamine hydrochloride to give compound **13b** (0.91 g, 91%) as a white solid (from ethyl acetate/petroleum ether).

^{1}HNMR (400 MHz, CD_3OCD_3): δ = 1.36 (s, 9H, CH$_3$ Boc), 2.61-2.64 (m, 2H, CH$_2$Dopa), 2.90-2.97 (m, 1H, βCH$_2$), 3.12-3.17 (m, 1H, βCH), 3.35-3.41 (m, 2H, CH$_2$ Dopa), 4.32-4.38 (m, 1H, αCH), 6.01 (br. d, *J* = 7.6 Hz, 1H, NH), 6.54 (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H, ArH Dopa), 6.73 (d, *J* = 2.0 Hz, 1H, ArH Dopa), 6.75 (d, *J* = 8.0 Hz, 1H, ArH Dopa), 7.20-7.31 (m, 6H, ArH Phe + NH), 7.78 (br. s, 2H, OH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 28.47 [C(*C*H$_3$)$_3$], 35.75 (CH_2 Dopa), 39.14 (βCH$_2$), 41.74 (CH_2 Dopa), 56.72 (αCH), 79.24 [*C*(CH$_3$)$_3$], 116.02 (CH), 116.55 (CH), 120.80 (CH), 127.13 (CH),

128.96 (2CH), 130.19 (2CH), 131.82 (C), 138.81 (C), 144.30 (C), 145.80 (C), 156.10 (C=O), 171.93 (C=O) ppm.

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{22}H_{29}N_2O_5$: 401.20765; found: 401.20798.

4.5.3. Synthesis of a hydroxyphenylamide of *N*-protocatechoyl dehydro-phenylalanine and phenylalanine

Synthesis of the trifluorate of dehydrophenyalanine 4-hydroxybenzylamide, Tfa.H-ΔPhe-NH-Bzl(4-OH), 14a

Procedure E was followed using compound **12a** (0.66 g, 1.800 mmol) to give compound **14a** (0.68 g, 99%) as a white solid (from diethyl ether).

^{1}HNMR (400 MHz, DMSO): δ = 4.18 (d, *J* = 6.4 Hz, 2H, CH_2 Bzl), 6.67 (d, *J* = 8.4 Hz, 1H, ArH), 7.00-7.05 (m, 3H, ArH), 7.12 (s, 1H, βCH), 7.19-7.30 (m, 5H, ArH), 9.03 (t, *J* = 6.4 Hz, 1H, NH), 9.29 (s, 1H, OH) ppm.

^{13}C NMR (100.6 MHz, DMSO): δ = 41.67 (CH_2 Bzl), 114.95 (2CH), 126.66 (CH), 128.25 (2CH), 128.73 (CH), 128.77 (2CH), 129.87 (2CH), 133.75 (C), 156.35 (C), 160.97 (C), 196.45 (C=O) ppm.

Synthesis of the trifluorate of phenyalanine dopamine, Tfa.H-ΔPhe-NH-Dopa, 14b

Procedure E was followed using compound **12b** (0.81 g, 2.000 mmol) to give compound **14b** in quantitative yield as a yellow oil.

^{1}HNMR (400 MHz, DMSO): δ = 2.48-2.58 (m, 2H, CH_2 Dopa), 3.22-3.27 (m, 2H, CH_2 Dopa), 6.40 (dd, *J* = 8.0 Hz, *J* = 2.0 Hz, 1H, ArH Dopa), 6.56 (d, *J* = 2.0 Hz 1H, ArH Dopa), 6.61 (d, *J* = 8.0 Hz, 1H, ArH Dopa), 7.00 (s, 2H, ArH Phe), 7.13 (s, 2H, ArH Phe), 7.18-7.32 (m, 6H, ArH Phe + βCH + NH + OH), 8.61 (t, *J* = 6.0 Hz, 1H, NH) ppm.

^{13}C NMR (100.6 MHz, DMSO): δ = 34.09 (CH_2 Dopa), 40.67 (CH_2 Dopa), 115.50 (CH), 115.91 (CH), 119.17 (CH), 126.67 (CH), 128.29 (2CH), 128.73 (CH), 129.75 (C), 129.85 (2CH), 133.82 (C), 143.61 (C), 145.10 (C), 160.87 (C), 196.38 (C=O) ppm.

Synthesis of the 4-hydroxybenzylamide of *N*-protocatechoyl dehydrophenylalanine, Bz(3,4-OH)-ΔPhe-NH-Bzl(4-OH), 15a

Procedure F was followed using compound **14a** (0.19 g, 0.500 mmol) to give compound **15a** (0.15 g, 75%) as a yellow oil.

^{1}HNMR (400 MHz, CD_3OCD_3): δ = 2.42-2.48 (m, 2H, CH_2 Bzl), 6.80 (d, *J* = 6.8 Hz, 2H, ArH), 7.13 (d, *J* = 8.4 Hz, 2H, ArH), 7.25-7.36 (m, 6H, ArH), 7.41 (s, βCH, ΔPhe), 7.55-7.60 (m, 1H, ArH), 7.76-7.78 (m, 1H, ArH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 43.06 (CH_2 Bzl), 115.67 (CH), 120.01 (CH), 125.08 (CH), 125.78 (CH), 125.82 (CH), 127.11 (CH), 127.25 (CH), 127.78 (CH), 128.25 (CH), 128.42 (CH), 128.69 (CH), 129.76 (CH), 129.85 (CH), 132.59 (C), 134.48 (C), 139.37 (C), 141.30 (C), 143.63 (C), 155.50 (C), 159.74 (C), 170.41 (C=O), 171.88 (C=O) ppm.

Synthesis of the trifluorate of phenyalanine 4-hydroxybenzylamide, Tfa.H-Phe-NH-Bzl(4-OH), 16a

Procedure E was followed using compound **13a** (0.74 g, 2.000 mmol) to give compound **16a** (0.66 g, 85.2%) as a white solid. (from methanol/diethyl ether). M.p. 204.0-205.0 °C.

^{1}HNMR (400 MHz, DMSO): δ = 2.94-3.02 (m, 2H, CH_2 Bzl), 3.96 (t, *J* = 7.2 Hz,1H, αCH), 4.09 (dd, *J* = 5.6 Hz, *J* = 8.8 Hz, 1H, βCH), 4.20 (dd, *J* = 5.6 Hz, *J* = 8.8 Hz, 1H, βCH), 6.66 (d, *J* = 8.0 Hz, 2H, ArH Bzl), 6.89 (d, *J* = 8.0 Hz, 2H, ArH Bzl), 7.19 (d, *J* = 6.4 Hz, 2H, ArH Phe), 7.26-7.34 (m, 3H, ArH Phe), 8.12 (br. s, 3H, NH_3), 8.66 (t, *J* = 5.6 Hz, 1H, NH), 9.34 (s, 1H, OH) ppm.

^{13}C NMR (100.6 MHz, DMSO): δ = 37.12 (βCH_2), 41.98 (CH_2 Bzl), 53.62 (αCH), 115.04 (2CH), 127.18 (CH), 128.11 (C), 128.57 (2CH), 128.85 (2CH), 129.48 (2CH), 134.88 (C), 156.50 (C), 167.52 (C=O) ppm.

Synthesis of the 4-hydroxybenzylamide of *N*-protocatechoyl phenylalanine, Bz(3,4-OH)-Phe-NH-Bzl(4-OH), 17a

Procedure F was followed using compound **16a** (0.19 g, 0.500 mmol) to give compound **17a** (0.131 g, 65%) as a light yellow solid. (from ethyl acetate/petroleum ether).

^{1}HNMR (400 MHz, CD_3OCD_3): δ = 2.95-3.08 (m, 2H, CH_2 Bzl), 4.17 (d, *J* = 6.0 Hz, 2H, βCH_2 Phe), 4.61-4.67 (m, 1H, αCH), 6.66-6.73 (m, 2H, ArH), 6.71 (d, *J* = 8.4 Hz, 1H, ArH), 6.99-7.01 (m, 2H, ArH), 7.14-7.31 (m, 7H, ArH), 8.15 (d, *J* = 8.4 Hz, 1H, NH), 8.36 (t, *J* = 6.0 Hz, 1H, NH), 9.30 (br. s, 1H, OH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 37.31 (CH_2 Bzl), 41.67 (βCH_2), 54.92 (αCH), 114.69 (CH), 114.94 (2CH), 115.23 (CH), 119.26 (CH), 125.27 (C), 126.12 (CH), 128.01 (2CH), 128.44 (2CH), 129.15 (2CH), 129.37 (C), 138.50 (C), 144.71 (C), 148.44 (C), 156.18 (C), 170.32 (C=O), 171.36 (C=O) ppm.

4.5.4. Synthesis of the 4-hydroxybezylamide of a *N*-acyl dehydrotripeptide, Npx-Ala-ΔPhe-Phe-NH-Bzl(4-OH), 18a

Procedure F was followed using Npx-Ala-ΔPhe-OH (0.051 g, 0.113 mmol) and compound **16a** to give compound **18a** (0.056 g, 70% after chromatography) as a white solid. (from acetone/diethyl ether). M.p. 231.0-232.0 °C.

^{1}HNMR (400 MHz, CD_3OCD_3): δ = 1.42 (t, *J* = 7.2 Hz, 3H, βCH_3), 1.53 (t, *J* = 6.8 Hz, 3H, CH_3 Npx), 3.05 (dd, *J* = 10.4 Hz, *J* = 4.0 Hz, 1H, CH_2 Bzl), 3.42 (dd, *J* = 4.0 Hz, *J* = 10.4 Hz, 1H, CH_2 Bzl), 3.89 (s, 3H, OCH_3), 3.90-3.92 (m, 1H, CH Npx), 4.22-4.28 (m, 2H, βCH_2 Phe), 4.41 (dd, *J* = 6.4 Hz, *J* = 8.4 Hz, 1H, αCH), 4.72-4.78 (m, 1H, αCH), 6.79 (d, *J* = 8.8 Hz, 2H, ArH Bzl), 7.05 (s, 1H, ArH), 7.07 (d, *J* = 2.4 Hz, 1H, ArH), 7.13 (d, *J* = 2.4 Hz, 1H, ArH), 7.16-7.34 (m, 12H, ArH), 7.54 (dd, *J* = 4.8 Hz, *J* = 4.0 Hz, 2H, ArH), 7.64 (d, *J* = 1.2 Hz, 1H, ArH), 7.72 (d, *J* = 8.4 Hz, 1H, NH), 7.83 (br. t, *J* = 6.0 Hz, 1H, NH), 7.93 (d, *J* = 4.0 Hz, 1H, NH), 8.23 (s, 1H, NH), 8.52 (s, 1H, OH) ppm.

^{13}C NMR (100.6 MHz, CD_3OCD_3): δ = 16.67 (βCH_3), 19.21 (CH_3 Npx), 37.99 (CH_2 Bzl), 43.07 (βCH_2), 46.60 (CH Npx), 52.21 (αCH), 55.58 (OCH_3), 56.26 (αCH), 106.45 (CH), 115.85 (2CH), 119.61 (CH), 126.75 (CH), 127.01 (CH), 127.02 (CH), 128.01 (CH), 129.10 (2CH), 129.27 (2CH), 129.53 (C), 129.57 (CH), 129.70 (2CH), 129.76 (C), 129.84 (2CH), 129.96 (CH), 130.02 (CH), 130.27 (2CH), 131.18 (C), 134.62 (C), 134.66 (C), 137.54 (C), 139.68 (C), 157.21 (C), 158.55 (C), 164.92 (C=O), 171.55 (C=O), 174.44 (C=O), 176.68 (C=O) ppm.

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{42}H_{43}N_4O_6$: 699.31826; found: 699.31835.

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